Chapter 3. Developmental Toxicity 1: Perinatal Manifestations

A summary of the conclusions regarding the evidence of a causal association between ETS exposure and perinatal manifestations from the 1997 OEHHA report and this update are provided below in Table 3.0. These findings are based on a weight of evidence approach.

Table 3.0 ETS and Pregnancy Outcomes: Comparison of OEHHA (1997) and Update

Outcome	# Studies 1997	# Additional Studies in Update	Findings OEHHA 1997 Evidence of causal association?	Findings Update Evidence of causal association?
BW	24	15	Conclusive	Conclusive (strengthened)
LBW	13	9	Conclusive	Conclusive (strengthened)
PTD	6	4	Suggestive	Suggestive (strengthened)
IUGR	5	7	Suggestive	Suggestive (strengthened)
SAB	5	2	Inconclusive	Inconclusive
Malformations	5	6	Inconclusive	Inconclusive

BW = birth weight, LBW = low birth weight, PTD = preterm delivery, IUGR = intrauterine growth restriction, SAB = spontaneous abortion

In summary, prenatal exposure to ETS has been shown to cause a decrease in birth weight and an increased risk of low birth weight. There is suggestive evidence of an association between ETS exposure and preterm delivery and intrauterine growth restriction.

3.0. Introduction

The detrimental effects of active smoking upon pregnancy are well documented and unequivocal, providing a framework for investigating the effects of environmental tobacco smoke (ETS) exposure upon reproduction. Maternal active smoking adversely affects fetal growth and is associated with decreased birth weight, small for gestational age babies, preterm deliveries (especially prior to 33 weeks gestation), placenta previa, placental abruption, spontaneous abortions, and fetal demise (Andres and Day 2000; U.S.DHHS, 2001).

Since the previous monograph, there have been three major developments in our understanding of active smoking and pregnancy that materially affect the evaluation of the effects of ETS on non-smoking pregnant women.

3.1. Mechanisms of Injury to Reproduction from Tobacco Smoke

It has been assumed that the main deleterious effect of active smoking has been due to nicotine and carbon monoxide in tobacco smoke. Nicotine's adverse effects have been thought to be due to its vasoconstrictive properties resulting in reduced maternal and fetal placental blood flow. (Quigley *et al.,* 1979) Human and animal studies indicate that this is probably not the only mechanism of nicotine toxicity upon pregnancy (Lambers and Clark, 1996), although it continues to be widely stated (Horta *et al.,* 1997). Nicotine functions as a neurotransmitter (acetylcholine) and nicotine's detrimental effects upon the fetus are probably due to the consequences of inappropriate stimulation of nicotinic cholinergic receptors (Dempsey and Benowitz, 2001; Slotkin, 1998).

Carbon monoxide is a potent fetotoxin (Koren *et al.,* 1991; Norman and Halton, 1990; Penney, 1996) which avidly binds to maternal and fetal hemoglobin and displaces oxygen. Fetal carboxyhemoglobin levels are higher than maternal levels (Bureau *et al.,* 1982) and carboxyhemoglobin has a half-life of 5 to 6 hours. Binding of carbon monoxide to hemoglobin adversely affects the release of the remaining oxygen molecules from hemoglobin. This detrimentally affects the transfer of oxygen across the placenta from the mother to the fetus, and the transfer of oxygen from the fetal blood to fetal tissue, resulting in chronic fetal tissue hypoxia (Longo, 1977). Whether the low levels of nicotine and carbon monoxide exposure associated with ETS exposure could alone account for the adverse outcomes attributed to ETS is not clear.

Tobacco smoke contains thousands of toxic chemicals including oxidative gases, heavy metals, cyanide, and carcinogens (Hoffmann *et al.,* 1997). Numerous studies have revealed a wide variety of molecular biologic differences between non-smoking pregnant women, their fetuses and newborns compared to active smokers and their progeny (Dempsey and Benowitz, 2001). Many of these differences are not due to nicotine or carbon monoxide exposure. Presently, the clinical significance of many of these differences is unknown, but the additive or synergistic sum of these alterations from nicotine, CO, and thousands of other chemicals may be responsible for the adverse reproductive outcomes associated with maternal smoking. The following are a few examples from a recent detailed review of this topic (Dempsey and Benowitz, 2001).

Active maternal smoking is associated with premature rupture of the chorio-amniotic membranes, especially prior to 33 weeks gestation, resulting in premature delivery (Meyer and Tonascia, 1977). The copper enzyme lysyl oxidase is important in the biosynthesis and maintenance of collagen, an important component of the chorio-amniotic membrane that surrounds amniotic fluid. Exposure to nicotine and/or tobacco smoke appears to reduce lysyl oxidase activity in hamster lungs (Osman *et al.,* 1985) and neonatal rat lung (Maritz *et al.,* 2000), and may well have a similar effect in the placenta. Impairment of placental lysyl oxidase may lead to premature rupture of membranes precipitating preterm delivery. Cadmium may impair lysyl oxidase by decreasing available copper due to induction of metallothionein (King *et al.,* 1997). It is known that copper levels are altered in mothers and fetuses of active smokers compared to non-smokers (Kuhnert *et al.,* 1993; Chambers *et al.,* 1994). Whereas in non-smokers, cadmium exposure is primarily through diet, in smokers the main source of cadmium is cigarette smoke, even in people who reside in proximity to a cadmium smelter (Lagerkvist *et al.,* 1993). Vitamin C, an antioxidant, is very important for the maintenance of the chorio-amniotic membranes. Low vitamin C levels are associated with preterm rupture of membranes and premature delivery (Casanueva *et al.,* 1993). Pregnant smokers have lower vitamin C levels than non-smokers, and this has been attributed to consumption of vitamin C by the oxidative gases in cigarette smoke as well as to reduced dietary intake (Schectman *et al.,* 1989; Klesges *et al.,* 1998). In addition, among children consuming equivalent amounts of vitamin C in their diets, ETS exposure has been associated with significantly ($p = 0.002$) lower plasma vitamin C levels (Preston *et al.,* 2003). Fibronectin, formed in the placenta and amnion, is thought to be important in intracellular adhesion and may play a role in pre-term delivery (PTD) (Shimizu *et al.,* 1992). Two volatile compounds in cigarette smoke, acrolein and acetaldehyde, individually inhibit fibronectin (Carnevali *et al.,* 1998). A rise in amniotic fluid levels of platelet activating factor (PAF) may be important in the initiation of labor. Cigarette smoking may contribute to preterm labor by its effect on PAF. Platelet activating factor is inactivated by PAF-acetylhydrolase (Narahara and Johnston, 1993). Components of cigarette smoke (other than nicotine, cotinine and CO) inactivate PAF-acetylhydrolase (Bielicki *et al.,* 2001). Reduced deactivation of PAF due to smoking would allow PAF to rise in amniotic fluid and precipitate labor. (Further information on the effects of ETS on platelet function are reviewed in the cardiovascular chapter.) These are examples of ways in which toxins in tobacco smoke may

contribute to premature rupture of membranes and/or premature delivery, and the same may be true for ETS exposure. Other differences between pregnant active smokers and pregnant non-smokers include alterations in estrogen levels, beta 1-glycoprotein, norepinephrine, vanillylmandelic acid, dopamine, human macrophage metalloelastase, epidermal growth factor, human placental lactogen, prolactin, human chorionic gonadotropin, prostacyclin, prostaglandin E2, prostaglandin F2a, phospholipase A2, and erythropoietin (Dempsey and Benowitz, 2001).

The picture that emerges from these data is that the deleterious effects of active smoking upon pregnancy may be due to a myriad of pathophysiologic processes acting additively or synergistically. Adverse reproductive outcomes are probably not due solely to the effects of one or two toxins in cigarette smoke. For example, in newborn infants, there is a statistically significant difference in the plasma levels of polychlorinated biphenyls and hexachlorobenzene between those born to non-smoking mothers exposed to ETS and those unexposed to ETS (Lackmann *et al.,* 2000). When the toxicity of cigarette smoke is viewed from the perspective of fetal exposure to hundreds or thousands of chemicals, it is much more biologically plausible that the sum of the toxins in ETS could materially affect pregnancy through a host of pathologic processes.

3.1.1. Gene-Environment Interactions

The ability to metabolize and eliminate drugs and toxins has significant variability in the population, part of which is due to genetic polymorphism of metabolizing enzymes. For example, it has been shown that occupational exposure to low levels of benzene is associated with a small decrease in the gestational age at birth when compared to an unexposed control group (Wang *et al.,* 2000). When the exposed and control groups were stratified by genotype for two drug metabolizing enzymes, CYP1A1 and GSTT1, mothers occupationally exposed to benzene who had the genotype CYP1A1 (AA) and GSTT1 (absent) had the greater decrease in gestational age compared to controls or benzene-exposed mothers with the genotype of CYP1A1 (Aa or aa) - GSTT1 (present). Among women who were unexposed to benzene, there was no effect of genotype on gestational age (GA) (Wang *et al.*, 2000).

Several gene interactions with active maternal smoking have now been reported (Hong *et al.,* 2001; van Rooij *et al.,* 2001). Important cigarette smoke carcinogens include polycyclic

aromatic hydrocarbons (PAH), arylamines, and N-nitrosamines. The phase one-enzyme arylhydrocarbon hydroxylase (CYP1A1) metabolizes PAH to highly reactive electrophilic intermediates, which in turn are converted to polar metabolites by conjugation with glutathione via glutathione-S-transferase (GSTT1) and excreted from the body. The effects of differences in the genotypes of these enzymes on two birth outcomes was examined in a case control study enrolling 207 PTD and/or low birth weight (LBW) infants, and 534 full-term non-LBW infants (Wang *et al.,* 2002). All infants were singletons without malformations. Among babies born to mothers who were non-smokers, the genotypes of the CYP1A1 enzyme and/or GSTT1 were not associated with decreased birth weight (BW). Maternal smoking was associated with a mean decrease in BW of 377 g (SE 89 g; $p<0.001$). When babies born to smokers were stratified by genotype, the CYP1A1 (AA) genotype was associated with a mean decrease of 252 g (SE 111 g; $p=0.02$) while the Aa or aa genotype was associated with a 520 g (SE 124 g; $p<0.001$) decrease. The presence of the GSTT1 genotype was associated with a 285 g (SE 99 g; $p=0.004$) decrease while absence of the genotype was associated with a 642 g (SE 154 g; $p<0.001$) decrease. There were 11 babies born to mothers with the CYP1A1 (Aa or aa) genotype and GSTT1 absent genotype, and their average birth weight reduction was 1,285 g (SE 234 g, $p<0.001$). These data suggest that there was an interaction between genotype and smoking with deleterious effects upon both BW and GA. These data demonstrated a very large effect of smoke exposure on birth weight associated with the ability to metabolize carcinogens in cigarette smoke. Similarly, these data indicate that it is biologically plausible that maternal ETS exposure may adversely affect pregnancy outcomes in selected groups based on genetic ability to metabolize chemicals in cigarette smoke.

3.1.2. Effects of Pregnancy upon the Biomarker Cotinine

In non-pregnant adult smokers, cotinine, the major proximate metabolite of nicotine, is a validated biomarker of smoking and correlates with the daily intake of nicotine from cigarette smoke much better than the count of cigarettes smoked per day (Benowitz, 1999). Studies of the effect of active smoking upon reproduction have found cotinine levels to correlate with adverse outcome measures in a dose-dependent manner. The levels of cotinine in saliva and blood are very similar, while the levels of cotinine in urine are approximately six times that of blood (Benowitz, 1999). There is good correlation between blood, saliva, and urine levels of cotinine.

The mean blood or saliva level of cotinine in ETS-exposed non-smoking adults in the U.S. is well below 10 ng/ml, usually in the neighborhood of 1 ng cotinine per ml (Pirkle, 1996). Blood cotinine levels for self-reported ETS-exposed and unexposed non-smokers greatly overlap and there is also some overlap with active smokers. The mean blood cotinine level for an ETS-exposed non-smoker has been reported as 0.8 ng/ml (Pirkle, 1996). In non-pregnant adults, the mean half-life of cotinine is between 17 and 20 hours and tends to remain at steady state from day to day. In non-pregnant adults, the blood cotinine level generally used to separate smokers from non-smokers is 10 ng/ml (Pirkle *et al.,* 1996; Rebagliato *et al.*, 1998).

Since publication of the previous monograph, we have expanded our knowledge of the effect of pregnancy upon the biomarker cotinine and the utility of cotinine as a biomarker during pregnancy. A recent study found the mean half-life of cotinine in pregnant women was 8.8 hours (95% CI 5.5; 12) compared to 16.6 hr for the same women 3 months postpartum (16.6 hr; 95% CI 14.8; 19) (Dempsey *et al.,* 2002). Gestational age was not found to affect the clearance of cotinine. The more rapid clearance of cotinine in pregnant women means that the cotinine levels in occasional and light smokers (<5 CPD) may fall into the range of non-smokers during periods of abstinence such as nighttime sleeping (Benowitz and Jacob, 1994). These data also explain the findings of Rebagliato, who found that the saliva cotinine was 3.5 ng/ml saliva per cigarette per day (CPD) during pregnancy and 9.9 ng/ml saliva per CPD postpartum (Rebagliato *et al.,* 1998). Based on these data, blood cotinine levels of 10 ng/ml in a non-pregnant woman and 3.6 ng/ml in a pregnant woman represent approximately equivalent smoke exposures. As a result, the cotinine blood or saliva levels of 10 ng/ml or higher that investigators have used to separate non-smoking pregnant women from active smokers are probably too high for pregnancy and would include light active smokers among their non-smokers. As a biomarker of exposure during pregnancy, a blood cotinine level below 3 ng/ml is probably a more suitable cut off to discriminate between maternal smokers and non-smokers.

A biomarker of exposure is needed because quantitating ETS exposure by history is very difficult. Urine levels of cotinine are approximately six times that of saliva or blood (Benowitz, 1999). This greater concentration relative to blood or saliva may allow for separation of non-smokers with no ETS, non-smokers with ETS and maternal smokers (Wang *et al.,* 1997). One advantage of urine is that cotinine may still be detectable in the urine even if it is below the level

of detection in blood or saliva. Presently, urine cotinine levels are probably the best available biomarker of ETS exposure during pregnancy. In a study of newborns of smoking mothers the mean concentrations of cotinine in their urine was 151 ng/ml while the sum of the concentrations of nicotine and 4 other nicotine metabolites was 745 ng/ml (Dempsey *et al.,* 2000). In a study of pregnant smokers, urine cotinine accounted for only 18.3% of the sum total of nicotine and its metabolites in the urine (Dempsey *et al.,* 2002). Methodologies are being developed for LC-MS-MS assays of nicotine and five metabolites (nicotine glucuronide, cotinine glucuronide, 3'-hydroxycotinine, and 3'-hydroxycotinine glucuronide) (Jacob *et al.,* 2002). It may be that the sum of nicotine metabolites in urine may serve as a superior dose-dependent biomarker for ETS exposure during pregnancy than blood cotinine.

Maternal and newborn hair levels of nicotine have also been used as a biomarker of ETS exposure during pregnancy, but there has been poor correlation between maternal and neonatal hair nicotine levels (Nafstad *et al.,* 1998). There are practical and methodological limitations to hair analysis. Some newborns are bald or nearly bald and so obtaining a sample may be difficult. Adult hair is highly variable as to thickness, color, and curl, which may affect nicotine deposition. Additionally, dyeing, bleaching, and perming hair may also affect the nicotine content. There appears to be, however, good correlation between maternal smoking histories and maternal nicotine hair levels (Eliopoulos *et al.,* 1996).

3.1.3. ETS Exposure in Pregnancy: the Association Between Self-Report and Cotinine

Studies of the effects of ETS exposure tend to rely heavily on maternal self-report. With the establishment of cotinine as a biomarker of ETS exposure along with the determination of levels that discriminate exposed and truly non-exposed pregnant women, it is possible to examine the association between self-reported ETS exposure and that indicated by serum cotinine levels.

A population-based sample of 680 pregnant women in California was used by DeLorenze *et al.* (2002) in a comparison of serum cotinine levels in blood taken during the mid-second trimester of pregnancy with the women's responses to an ETS exposure question asked around the time of delivery. The question on ETS specifically asked how many hours per day, during the fourth and fifth months of the pregnancy, the mother spent indoors with other people who were smoking at home, work and other places. The assay used for cotinine was highly sensitive with a

limit of detection of 0.05 ng/ml. Multivariate analysis was used to estimate the mean change in log serum cotinine as a function of hours per day of ETS exposure at all sites, combined and separately.

After controlling for marital status, payment source for prenatal care, language spoken at home, and tea consumption, the analyses showed that self-reported total hours per day of ETS exposure was a significant predictor of (log) serum cotinine when modeled as a function of a cubic polynomial ($R^2 = 0.27$). The data were also predictive when coded categorically as any hours per day of ETS exposure at any site ($R^2 = 0.17$).

Based on responses to the ETS question, 72% (n = 490) of the participants reported no ETS exposure. However, the corresponding cotinine values for this group indicated a wide range of ETS exposures (0.001-3.67 ng/ml). Regression analysis incorporating demographic variables indicated that the reportedly unexposed women with higher cotinine levels were more likely to be unmarried and of lower socioeconomic status. These data suggest that studies of ETS exposure in pregnant women that rely on an hours-per-day ETS exposure question likely misclassify some portion of ETS-exposed women as non-exposed. As a result, the association of ETS exposure with pregnancy outcomes would be under-estimated in such studies.

In a related article conducted in the same population of pregnant women, Kaufman *et al.* (2002) examined the agreement between a question about the number of smokers in the household and serum cotinine levels. The results showed that even when no ETS exposure was reported at home, at work or in other places, serum cotinine levels were twice as high in women reporting living with one or more smokers (0.08 ng/ml, 95% CI = 0.05, 0.13, $p < 0.002$) as compared to women reporting no smokers in the home (0.04 ng/ml, 95% CI = 0.04, 0.05). Although the authors acknowledged the result may be due to ETS exposure in other places that was not adequately measured in this study, it was proposed that the higher cotinine levels may have resulted from exposure to nicotine emitted from a smoker's clothes or hair. Nicotine from ETS is deposited on surfaces such as walls, carpets, and clothes, and can be emitted back into the air from these surfaces. Low levels of nicotine have been measured in the air in rooms where smoking had occurred in the past, and urinary cotinine concentrations have been measured in subjects exposed to a room where smoking occurred in the past (Nelson *et al.*, 1991). This

component of ETS exposure may help to explain some of the variability between serum cotinine concentrations and questionnaire data of exposure, especially where levels of exposure is low.

3.2. Fetal Growth and Preterm Delivery

3.2.1. Epidemiological Studies

This section includes studies published since the previous monograph that investigate the following topics: birth weight (BW), low birth weight (LBW), small for gestational age (SGA), small for dates (SFD), intrauterine growth retardation (IUGR), preterm delivery (PTD), spontaneous abortion (SAB), and pregnancy wastage. There has been an attempt to extract the same types of data from each article; specifically, the total number of subjects enrolled, the number of subjects in each cohort, and the overall differences (significant or non-significant) between the cohorts prior to stratifying the data into subgroups based on maternal characteristics or types of ETS exposure. We have also tried to identify confounders and covariates, as well as studies that have included maternal active smokers in their analysis of ETS exposure. Studies presenting data on the effects of ETS on fetal growth retardation, measured as IUGR and SGA are summarized in Figure 3.3.

Several issues regarding covariates and confounders need to be considered. The most important determinant of BW is gestational age (GA). GA is an extremely important covariate of studies of ETS exposure and BW. Between 36 and 40 weeks of gestation, the fetal weight increases by approximately 100 g per week, so a one-week difference or even a three to four day difference in mean GA may result in a mean difference in BW of 50-100 g. This magnitude of difference in BW to GA is greater than or similar to the BW decrements reported by some authors to be associated with ETS exposure. Studies that include GA in their models will be given greater weight in the discussions and conclusions.

A confounder of studies of ETS exposure is maternal active smoking. There are several important considerations. The first is inadvertent inclusion of active smokers in the cohorts of non-smoking pregnant women. This may occur if active smokers self-identified themselves as non-smokers. Or it may occur if inclusion is based on biomarkers. Because of the increased clearance of cotinine during pregnancy, it is possible for the cotinine level of a light smoker (2-3

CPD) to fall to very low levels between the time of the last cigarette smoked and the time of sampling.

In addition, non-smokers and smokers have been shown to have statistically significant differences in their lifestyles (Koo *et al.,* 1988; 1997). This is even more pronounced when both parents smoke. These differences include time of entry into prenatal care, illicit drug use, alcohol consumption, socioeconomic status, maternal age, marital status, and parental education; and these lifestyle factors have also been associated with adverse reproductive outcomes. The risk factors with greatest magnitude of effect upon BW are a prior history of low birth weight or pre-term delivery. Other important risk factors include: ethnicity; maternal pre-pregnancy weight or body mass index, and maternal weight gain during pregnancy; maternal height; and parity. These factors are adjusted for in many of the newer epidemiological studies.

Table 3.1 ETS and Fetal Growth, Preterm Delivery and Birth Weight.

Reference Country	Study description	Smoke exposure measure	Findings and OR (95% CI)	Comments
Jedrychowski & Flak, 1996 Poland	Retrospective study of cotinine, smoke exposure and birth weight. 1007 non-smokers.	Maternal passive only Maternal passive only	Change in birth weight -57.9 g (p=0.004) OR low birth weight 1.46 (0.83; 2.6)	ETS significantly decreased BWs but not OR for LBW. However, cotinine cutoff of 25 ng/ml would mix light and non-smokers.
Ahluwalia et al.1997 US	Retrospective study of the interaction of age and ETS on birth weight and premature births. n=17,412	Maternal passive only <30 yr old >30 yr old <30 yr old >30 yr old <30 yr old >30 yr old	OR low birth weight 0.97 (0.76; 1.23) 2.42 (1.51; 3.87) OR preterm birth 0.92 (0.76; 1.13) 1.88 (1.22; 2.88) Change in birth weight 8.8 g (43.7; -26.1) -90 g (0.8; -180.9)	ETS during pregnancy significantly increased risk of LBW and preterm delivery in non-smoking women over 30 yrs old, but not in younger women.
Horta *et al.* 1997 Brazil	Retrospective study n=5,166	Maternal passive only Maternal passive only Maternal active Paternal smoking	OR low birth weight 1.18 (0.94; 1.48) OR pre-term delivery 1.25 (0.99; 1.57) 1.02 (0.80; 1.29) OR IUGR 1.33 (1.05; 1.68)	Significance of results hard to evaluate as ETS was not quantified, and little data were given on BWs and sizes of exposure groups.
Lodrup Carlson et al. 1997 Norway	Prospective cohort study of asthma. Birth weight and ETS data. n=803.	Maternal passive only No ETS ETS exposed	Birth weight (SD) 3.6 kg (49 g) 3.5 kg (46 g) p=0.04	Significantly lower BW with ETS but values unadjusted for gestational age or other confounders.
Peacock et al. 1998 UK	Prospective study of maternal plasma cotinine and birth weight. n=703 Also meta-analysis	Maternal passive only Meta-analysis +ETS	Change in birth weight -6.7 g (-84; 97) -31 g (-44; -19)	BW adjusted for gestational age, maternal height, parity and gender. Meta analysis of 11 studies found significant decrease in BW with ETS.

BW birth weight; CPD cigarettes per day; HC head circumference; IUGR intrauterine growth restriction; L body length; LBW low birth weight; SES socioeconomic status; SGA small for gestational age.

Table 3.1 ETS and Fetal Growth, Preterm Delivery and Birth Weight (continued).

Reference Country	Study description	Smoke exposure measure	Findings and OR (95% CI)	Comments
Luciano *et al.*, 1998 Italy	Prospective cohort study. Maternal passive and light active smoking on fetal growth.	Maternal None ETS only Light active	BW Placenta wt 3604 g 603 g 3351 g 553 g 3378 g 541 g p< 0.013 p<0.001	Significantly lower BW, placental weight, cranial circumference, length, etc. with passive and active smoking. Limited confounder control.
Dejin-Karlsson et al. 1998 Sweden	Prospective study. ETS and risk of small-for-gestational-age infants. n=826	Maternal exposure Non-smoker + ETS Active smoker + ETS	OR SGA 3.9 (1.4; 10.7) 6.0 (2.1; 17.5)	ETS, as dichotomous variable, raised risk of SGA births. ORs adjusted for maternal age, weight, height, nationality and education.
Nafstad et al. 1998 Norway	Case-control study of small-for-gestational-age and hair nicotine. 58 cases; 105 controls	Maternal exposure Maternal hair nicotine < 0.75 µg/g 0.75-4 µg/g > 4 µg/g	OR small-for-gestational age 1 (reference) 3.4 (1.3; 8.6) 2.1 (0.4; 10.1)	Increased risk with maternal hair nicotine > 0.75 µg/g. Apparent lower risk at >4 µg/g likely due to small number of individuals in this category. Neonatal hair nicotine not correlated with outcome.
Hanke et al. 1999 Poland	Retrospective study of birth weight and ETS in non-smokers. n=1751	Maternal passive only ETS > 7 hr/d	Change in birth weight -100 g (no CI given)	BW decrease became non-significant after adjustment for gestational age.
Windham 1999 US	Retrospective study of ETS and birth weight. N = 992	Maternal passive only	Low term birth weight 1.8 (0.64; 4.8) SGA 1.4 (0.79; 2.5)	SGA ORs adjusted for multiple confounders but BW adjusted only for race, alcohol and caffeine consumption.
Haug et al. 2000 Norway	Retrospective study on birth weight and parental smoking n=22,883	Parental smoking Active maternal only Active paternal only Both	Birth weight decrease 153 g (128; 178) 1 g n.s. 201 g (185; 218)	Statistically non-significant decrease in BW with maternal exposure to ETS unless she is also an active smoker.
Matsubara et al, 2000 Japan	Prospective population-based cohort study of smoke exposure and birth outcomes. n=7,411	Maternal passive Active paternal only Any passive	OR IUGR* 0.95 (0.72-1.26) Birth weight decrease 19 g p<0.05	Significant decrease in BW but statistically non-significant decrease in IUGR with maternal exposure to ETS.

BW birth weight; CPD cigarettes per day; HC head circumference; IUGR intrauterine growth restriction; L body length; LBW low birth weight; SES socioeconomic status; SGA small for gestational age.

Table 3.1 ETS and Fetal Growth, Preterm Delivery and Birth Weight (continued).

Reference Country	Study description	Smoke exposure measure	Findings and OR (95% CI)	Comments
Hruba & Kachlik, 2000 Czecholslovakia	Retrospective study of ETS and birth weight. n=1,097	Maternal passive only Never smokers +ETS Former smokers +ETS Former smokers -ETS	Change in birth weight -65 g +2 g +32 g	ETS apparently decreased BW for never smokers and modified weight gain in former smokers but no statistical analysis provided.
Windham et al. 2000 US	Prospective study of ETS and birth weight in non-smokers. n=3646	Maternal passive only Moderate ETS High ETS > 12 hr ETS/day	Change in birth weight +0.68 g +8.2 g -88 g	Study group comprised women in pre-paid plan seeking prenatal care; not representative of general population. All birth weight CIs included 0
Jaakkola et al. 2001 Norway	Cohort study of ETS and hair nicotine on birth weight n=389	Maternal exposure per µg nicotine/g hair ETS home ETS work	Birth weight -0.91 g (-20;+18) -99 g (-273;+75) -101 g (-258;+56)	No significant association of nicotine or ETS with birth weight but no control for gestational age.
Kukla et al. 2001 Czechoslovakia	Prospective study of smoke exposure and birth outcomes. n=4,530 1,178 ETS exposed 2,987 no exposure 365 active smokers	Maternal exposure passive <15 CPD passive >15 CPD active <10 CPD	Neonate parameters -4 g BW; +0.01 cm L +0.11 cm HC -74 g BW; -0.34 cm L +0.01 cm HC -79 g BW; -0.48 cm L -0.28 cm HC	High ETS similar to active smoking on birth weight, body length and head circumference but data not adjusted for gestational age, parity, SES, maternal height or weight, or other predictors of pregnancy outcome.
Kharrazi 2001 US	Prospective study of maternal serum cotinine and birth outcomes. n=2815	Maternal cotinine – 1.0 ng/ml >1.0 ng/ml >0.05 ng/ml	Change in birth weight -30 g -100 g OR adverse outcome 1.36 (1.07; 1.72)	Significant increases in adverse birth outcomes associated with maternal serum cotinine. ORs adjusted for maternal age, ethnicity, parity, infant gender, gestational age, insurance.
Dejmek et al. 2002 Czechoslovakia	Retrospective study of effects of active and passive smoking on birth outcomes n=8,624	Maternal passive only	OR low birth weight 1.51 (1.02; 2.26) OR IUGR 1.08 (0.82; 1.43)	ETS, defined as passive exposure to 5 or more cigarettes/day, significantly raised risk of low birth weight but not IUGR.

BW birth weight; CPD cigarettes per day; HC head circumference; IUGR intrauterine growth restriction; L body length; LBW low birth weight; SES socioeconomic status; SGA small for gestational age.

Table 3.2 ETS and Spontaneous Abortion

Reference Country	Study description	Smoke exposure measure	Findings and OR (95% CI)	Comments
Chatenoud et al. 1998 Italy	Case-control study of parental smoking and spontaneous abortion n=354	Maternal exposure Paternal smoking	Spontaneous abortion OR 0.8 (0.7; 1.0)	Non-significant effect of paternal smoking on spontaneous abortion but maternal smokers included with non- smokers in analysis of paternal effects.
Windham et al. 1999 US	Prospective study of ETS and spontaneous abortion. n=4,209	Maternal passive only	Spontaneous abortion OR 1.01 (0.8; 1.27)	Study group comprised women in pre-paid plan seeking prenatal care; may not represent general population.

Jedrychowski and Flak, 1996. This is a Polish retrospective study of ETS and BW of 1,165 school age children; half recruited from a polluted area of Krakow and half recruited from a clean area of Krakow. Data were available for 1,115. The mothers were interviewed for active and passive smoking during the pregnancy of the child in the study. Birth weight, GA at birth, and other perinatal characteristics were also obtained from the mother. During the pregnancy of interest, there were 452 non-smokers without, and 512 with exposure to ETS. Among smokers, 23 had no ETS and 135 were exposed. The crude mean decrease in BW for babies of non-smokers exposed to ETS was 73 g. After adjusting for GA as reported by the mother, the effect of ETS exposure upon the birth weight of babies born to non-smokers was 57.9 g (SE 31; p=0.004; 95% CI not reported). The OR of delivering an LBW baby for non-smokers with ETS was 1.46 (95% CI 0.83; 2.6).

Data were presented for a validation study of the sensitivity and specificity of plasma cotinine to identify active smokers in 158 pregnant women. A plasma cotinine level of 25 ng/ml was used to separate smokers and non-smokers. This is a high plasma cotinine threshold, most likely allowing inclusion of active smokers. Nevertheless, based on the 25 ng/ml criterion, the authors found a significant misclassification (false negative) rate of 57% of ETS-exposed women as non-exposed. Adjustment of the ORs for misclassification would substantially raise the risk estimates.

Ahluwalia et al., 1997. This is a study of ETS and birth weight data for 17,412 singleton births of low-income women reported to the CDC and Prevention Pregnancy Nutrition Surveillance System for the States of Arizona and North Dakota from 1989 to 1994. Home ETS exposure was self-reported as a yes/no response. Active cigarette smoking was defined as a yes/no response to having smoked any number of cigarettes asked at their initial prenatal care visit. Among the 17,412 mother/infant pairs, 3,817 were smokers of whom 67% were also exposed to ETS. Among the 13,497 non-smokers, 21.2% were exposed to ETS. The data were also stratified by the age of the mother because maternal age showed an obvious effect in the descriptive statistics. Among non-smokers less than 30 years of age, there was no difference in their babies' BWs between ETS exposed and ETS unexposed. After the age of 30, mean BW was 90 g lower in the offspring of non-smokers exposed to ETS (95% CI -0.8; 181) compared

to non-exposed nonsmokers. Among offspring of smokers, BWs were lower for those exposed to ETS with the greatest effect among smokers over 30 years of age.

Maternal non-smokers under the age of 30 did not have a significant increase in risk of LBW, SGA or PTD associated with ETS exposure. However, offspring of non-smokers over the age of 30 did have a significant increase in the risk of LBW and PTD after controlling for ethnicity, education, marital status, parity, geographic location, altitude, alcohol use, weight gain and pre-pregnancy BMI. For non-smokers over 30 years of age who were exposed to ETS, the OR for LBW was 2.42 (95% CI 1.51; 3.87), and for PTD the OR was 1.88 (95% CI 1.2; 2.88). For maternal smokers, ETS exposure was not associated with an additional increase in the OR for LBW, SGA, and PTD. However, there was an increase in the adjusted OR for LBW (OR = 1.39, 95% CI 1.0; 1.93) if the mother was under 30 years of age, smoked and was exposed to ETS. .

The study population included only low-income women. The relationship between ETS exposure and these outcomes may differ by socioeconomic status. Also the intensity and duration of ETS exposure was not recorded and it is not known how this may have differed between the age groups possibly contributing to the apparent differential effects with age.

Wang et al., 1997. This is a prospective Boston study of gene-environment interactions in maternal smokers. The cohort included 740 pregnant women enrolled prior to 20 weeks GA of which 410 were non-smokers with no ETS and 73 with ETS. Maternal smokers numbered 257. Urine and plasma samples were obtained at each post-natal care visit and analyzed for cotinine. Urine cotinine was corrected for creatinine. Parental smoking status and ETS exposure was obtained by interview. Mean urine cotinine level for non-smokers with no ETS was 20 ng/ml corrected for creatinine (95% CI 18.4; 21.6), while for those with ETS it was 41 ng (95% CI 35; 47) ($p<0.001$). The urine cotinine levels for the active smokers were generally above 1000 ng. At birth, the umbilical cord cotinine level correlated with both maternal serum cotinine ($r = 0.91$, $p<0.001$) and maternal urine cotinine ($r = 0.72$, $p<0.001$). Compared to non-smokers, babies of mothers who smoked daily had a mean BW that was 257 g lower, were 1.2 cm shorter, and had a 0.5 cm decrease in head circumference. For mothers who intermittently smoked, there was a mean decrease in their newborns' BW of 56 g, but the birth length and head circumference were similar to babies of non-smokers. After adjustment, each 1000 ng increase in urine cotinine

concentration was associated with a 59 ± 9 g decrease in BW ($p < 0.01$), 0.25 ± 0.05 cm decrease in birth length ($p < 0.01$), and a 0.12 ± 0.03 cm decrease in head circumference ($p < 0.01$). The authors stated that there was a small but detectable negative effect on BW, birth length, and head circumference when the maternal urinary cotinine level was 31-100 ng cotinine/mg creatinine in comparison to those with urine levels below 31 ng. "These results were suggestive" that ETS exposure of non-smokers affects fetal growth.

There are other concerns with this study. The participation rate was on the low side (75%) and no comparison with the women who did not participate was given, nor were the reasons for their exclusion. The authors' selection of urine cotinine levels of <31 ng/ml for the reference group is problematic since this level may include active smokers. In addition, the limit of detection of 3 ng/ml may be too high to discriminate truly non-exposed from exposed individuals. This study is not included in the tables.

Horta et al., 1997. This is a retrospective Brazilian study of 5,166 live singleton babies without malformations. Mothers were interviewed during the birthing hospitalization regarding their smoking habits and if their partner was a smoker. Among the mothers, 65.2% were non-smokers and 57% of their partners were non-smokers. No quantification of ETS exposure was done, nor were the sizes of the various cohorts given (non-smokers with and without ETS, smokers with and without ETS). Odds ratios were adjusted for social class, prior LBW, maternal height, maternal pre-pregnancy weight and prenatal care. Few birth weight data were reported. In the analysis of the effects of paternal smoking, the ORs were adjusted for maternal smoking. Babies born to mothers whose partner smoked had a 30 g decrease in BW ($p<0.05$). The adjusted OR for LBW if the partner smoked was 1.18 (95% CI 0.94; 1.48). The adjusted OR for pre-term delivery if the partner smoked was 1.25 (95% CI 0.99; 1.57); this was greater than the adjusted OR for pre-term delivery if the mother was a smoker during the whole pregnancy (OR 1.02, 95% CI 0.80; 1.29). The adjusted OR for IUGR if the father was a smoker was 1.33 (95% CI 1.05; 1.68). A strength of this study was the large number of live births. However, there was no quantification of ETS exposure as the only history elicited was maternal and paternal smoking status.

Lodrup Carlsen et al., 1997. This Norwegian study examined lung function in newborns and the association between maternal smoking, both active and passive, with newborn tidal flow-volume ratio and compliance of the respiratory system. A cohort of 3,754 newborns was established in Oslo, Norway, to prospectively study asthma. This study reports data for 803 healthy neonates with BWs >2,000 g, who underwent pulmonary function testing. ETS exposure in the mother did not appear to have an effect upon the pulmonary functions studies in the newborn. Birth weight data were also collected between January 1992 and March 1993. Questionnaire data were used to determine smoking status. The mother was classified as exposed to ETS based on the presence of daily smoking by a family member. The mean BW of 483 newborns of non-smokers with no ETS exposure was 3.6 kg (SD 0.49 kg). For mothers with ETS exposure, the mean BW was 3.5 kg (SD 0.46 kg); this was a significant difference from unexposed ($p=0.04$). For active smokers, the mean birth weight of their babies was 3.4 kg (SD 0.49 kg); also different from unexposed ($p <0.001$). The focus of this study was not BW, and BW data were not adjusted for covariates and confounders such as GA. Thus it is not known whether the changes in BW associated with ETS exposure would remain following adjustment.

Peacock et al,. 1998. This prospective study of women booking for prenatal care between 1982 and 1984 was conducted in London. The goal was to investigate whether maternal plasma cotinine levels, determined three times during pregnancy, were a better predictor of birth weight deficits from active smoking than a count of the CPD corrected for nicotine yield of the cigarette. A subsidiary goal of the study was to look at the relationship between cotinine levels and BW in maternal non-smokers whose smoking status was validated with a cotinine level. Of 1,860 pregnant women enrolled, 1,254 had all data elements collected including plasma cotinine levels. The plasma cotinine level, separating smokers and non-smokers, was 15 ng/ml. Histories of active smoking were obtained by trained interviewers. Passive smoking data was obtained by the question, "Does anyone else in the house smoke?" Among self-reported smokers, the data reported here supports previous work by Haddow and Bardy (Bardy, Seppala, *et al.* 1993 #175), which found that maternal cotinine levels were a better predictor than maternal CPD of BW deficits associated with active smoking. Among non-smokers, 283 reported ETS exposure at home, while 420 were reportedly ETS-unexposed. Almost all non-smokers reporting ETS exposure had cotinine levels that fell below 2.5-ng/ml plasma. Non-smokers were divided into

quintiles based on cotinine levels (0-0.180, 0.181-0.291, 0.292-0.480, 0.481-0.795, ≥ 0.796 ng/ml). Smokers with ETS exposure had higher cotinine levels than non-smokers but there was substantial overlap in levels.

There was a mean 73 g decrease in BW in babies born to ETS-exposed mothers in the highest continine quintile compared to the lowest (95% CI -28; 174). But after adjusting for gestational age, maternal height, parity, and sex of newborn, the decrease dropped to 6.7 g (95% CI -84, +97). Although this study lacked sufficient power to be conclusive, there was evidence that a reduction in cotinine levels, especially early in pregnancy, partially mitigated the effects of ETS on BW.

The authors also conducted a meta-analysis of 11 studies, including the data reported here and found a pooled estimate of difference in mean BW of -31 g (95% CI -44; -19) between ETS-exposed and ETS-unexposed. They suggested that studies showing a large effect of maternal ETS exposure upon BW did not correct for gestational age.

Luciano et al., 1998. This was an Italian prospective cohort study of the effects of maternal passive and light active smoking on intrauterine growth and body composition in 112 neonates born after normal pregnancies. Questionnaires were used to assess maternal smoke exposure (active and passive; at home and at work) prior to and during pregnancy, paternal smoking during pregnancy, maternal weight gain, alcohol and drug use, placental and birth weights, and paternal height and weight. After exclusion of women with gestational diabetes, alcohol consumption, drug addictions, first trimester infections, and exposure to radiation or teratogens, the remaining 112 mother-infant pairs were divided into three smoke exposure groups: nonsmokers with no ETS exposure; nonsmokers with significant ETS exposure (≥ 20 CPD); light active smokers (<10 CPD). Anthropometric measurements were taken within 24 hours of birth.

Compared to newborns of women with no smoke exposure, intrauterine growth was significantly lower in newborns of women with either passive or light active smoke exposure ($p<0.001$), but not significantly different between passive and active smokers. All auxometric measures (including birth and placental weights, fat mass, cranial circumference, height and other

measures) were lower in children of women exposed to either passive or light active smoking compared to children of non-exposed women. The differences in individual measures were statistically significant for most measures ($p = < 0.04$).

This is a relatively small study with no apparent control for potential confounders such as diet. No biochemical assessment of ETS exposure was made and the ETS-exposed group included only those with exposure to ≥20 CPD. While a dose-response effect cannot be demonstrated in this study, the data indicate that heavy passive smoke exposure and light active smoking have comparable deleterious effects on intrauterine growth.

Dejin-Karlsson et al., 1998. This is a Swedish study of 826 nulliparous women delivering singleton births in one city during a one-year period. Data were collected at the first prenatal care visit where a single yes/no question assessed ETS exposure at home or work. Routine ultrasound examinations, performed in 97.6% of the women at 16-18 weeks and at 32 weeks of gestation, were used to date pregnancies and assess fetal growth. Babies with BW two standard deviations below the population-specific GA-related mean were classified as SGA. Of the 826 mothers analyzed for the effects of ETS on SGA, 243 were smokers and 530 were ETS-exposed. Fifty five babies were small for gestational age (SGA), 11 babies were born prematurely, and 26 babies had BW below 2,500 g. Among non-smokers there were 240 without and 323 with exposure to ETS. There were 243 maternal smokers, 32 of whom were unexposed to ETS. Active smokers were included in the analysis of the effect of ETS exposure on fetal growth. The adjusted OR (maternal age, height and weight, nationality, and maternal education) for SGA babies delivered by a non-smoker exposed to ETS was 3.9 (95% CI 1.4; 10.7). The authors also found an increase in the risk of a smoker delivering a SGA baby if she was ETS-exposed (OR 6.0, 95% CI 2.1; 17.5).

One of the strengths of this study is the use of ultrasound measurements and population-specific growth curves in estimating SGA. This study did not evaluate the intensity or duration of ETS exposure but did include ETS exposure at work as well as at home. Participants were seen at both public and private clinics suggesting a potentially broad range of socioeconomic status for which there was no adjustment in the analysis, although there was adjustment for maternal education, which is correlated with SES.

Nafstad et al., 1998. This is a Norwegian case control study of 58 SGA babies (BW ≤ 90% of GA-corrected BW), and 105 controls, all born after 28 weeks gestation and excluding malformed babies or babies that required intensive intervention after birth. Data collection and maternal interview occurred within 30 hours after delivery. Maternal smoking status and ETS exposure was determined for each trimester. Nicotine was determined in maternal and neonatal hair samples. The limit of detection was 0.01-μg/g hair, with a 15 mg hair sample; if the level was less than the limit of detection, it was reported as 0. The smoking status of the mothers of SGA babies was: 22 non-smokers with no ETS; 17 non-smokers with ETS; 10 smokers of <10 CPD; and 9 smokers of >10 CPD. The smoking status of the mothers of controls was: 48 non-smokers with no ETS exposure; 37 non-smokers with ETS; 16 smokers of <10 CPD; and 6 smokers of ≥10CPD. Nicotine was detected in all maternal hair samples and the levels in smokers were 7-9-fold higher than in non-smokers. Four of 68 non-smokers without ETS and 5 of 54 non-smokers with ETS had nicotine hair levels above the 25th percentile for smokers. Otherwise, over half of the maternal nicotine levels in non-smokers with and without ETS had levels below the lowest level detected in active smokers. ETS-exposed non-smokers had a slight but non-significant increase in median nicotine hair levels. Neonatal hair samples did not show a similar trend between smokers and non-smokers exposed or unexposed to ETS. Maternal and neonatal hair nicotine levels did not correlate ($r = -0.03$, $p = 0.78$).

Based on maternal report, the OR for an SGA baby for non-smokers exposed to ETS was 1.0 (95% CI 0.4; 2.1) compared to no ETS. For calculations of risk based on nicotine levels, hair nicotine of <0.75 μg/g was the referent. For non-smokers with nicotine levels between 0.75 and 4 μg/g, the OR for SGA was 3.4 (95% CI 1.3; 8.6). Among non-smokers with nicotine hair levels above 4 μg/g, the OR for an SGA baby was 2.1 (95% CI 0.4; 10.1). However, this estimate was based on only three SGA babies. Based on self-report, the OR for an SGA baby was not elevated, but when non-smokers were stratified by maternal nicotine hair levels, there was a significant increase in the OR if the hair level was above 0.75 μg/g. Either non-smokers had more ETS exposure than they realized or they were light or occasional active smokers, or both.

A strength of this study is the objective measure of ETS exposure through hair nicotine analysis. This approach worked well for maternal hair, but insufficient hair was available for the analysis

from some neonates (43% of cases, 37% of controls). This problem was exacerbated by the small size of the study and may have contributed to the lack of correlation between maternal and neonatal hair nicotine levels. Nevertheless, measured as hair nicotine, ETS exposure was associated with an increased risk of SGA babies.

Hanke et al., 1999). This is a Polish Study of 1,751 rural and urban non-smoking mother/infant pairs. Mothers were interviewed within days of birth by physicians about their exposure to ETS. There were 827 mothers with ETS exposure, 924 without. Compared to no ETS, mothers with ETS exposure were less educated, shorter, had fewer prenatal visits, more were unmarried, and more resided with a smoker. There was an approximately 100 g decrease in BW of the 174 babies born to mothers exposed to 7 or more hours of ETS per day when compared to the 924 babies born to unexposed mothers after adjusting for maternal height and age. But, after adjusting for GA, there was no significant difference in BW between babies of ETS exposed and unexposed women. However, the effects of ETS on BW may be mediated in part by a shortening of the pregnancy. A significant excess risk of PTD among mothers exposed to ETS for 7 hr/d was seen in the authors' multivariate analysis (OR 1.86, 95% CI 1.05; 3.45). ETS appeared not to significantly affect the incidence of SGA babies.

Windham et al., 1999. For this California retrospective study, the study population of 992 smokers was the control population from a study of spontaneous abortions conducted between 1986 and 1987 (Windham *et al.*, 1992). Mothers were interviewed by telephone on average six months after delivery regarding maternal ETS exposure for three months prior to pregnancy and during the first half of the pregnancy. Paternal smoking habits were also ascertained for the same time interval. Women were considered to be ETS exposed if they regularly spent one or more hours per day in a room where someone was smoking. SGA was defined as BW less than the tenth percentile for GA at each week of gestation for weeks 24-44. LBW babies were defined as those weighing less than 2500 grams. Multivariate regression models used to examine the effects of ETS on mean BW were adjusted for GA, maternal age, education, parity, marital and employment status, hypertension, race, alcohol consumption, and caffeine consumption. In the logistic regression analysis of LBW and ETS exposure, only the last three variables were included as the other variables were found not to confound the association. On average, babies born to ETS exposed mothers weighed 34 g more (95% CI -43; 111) than those

of ETS unexposed mothers. After adjustment for covariates, including GA, this estimate decreased to 13.8 g (95% CI –53.8; 81.4) with wide confidence intervals that include no effect. However, this report included a meta-analysis of studies examining ETS and BW differences. Among the eight studies considering ETS exposure from all sources and providing adjusted estimates for BW differences, the pooled mean decrement in BW was –24.0 g (-39.3; -8.6); a significant decrement in weight. The adjusted OR for an LBW baby was 1.0 (95% CI 0.52; 2.1). The adjusted OR for a term LBW baby was 1.8 (95% CI 0.64; 4.8) and the adjusted OR for a SGA baby was 1.4 (95% CI 0.79; 2.5).

Haug et al., 2000. This is a Norwegian retrospective study that relies upon maternal recall. In the original study, the primary outcome of interest was SIDS. Postal questionnaires were sent in 1992 to mothers of singleton births, whose babies had no congenital anomalies, and were alive at 1 year of age. The survey years were 1970, 1975, 1980, 1985, 1989, 1990, and 1991; 34,799 questionnaires were sent out and 22,883 were returned. Smoking habits were recorded as yes/no to maternal smoking and yes/no to paternal smoking. Birth weight increased with maternal age for non-smokers regardless of paternal smoking status. Birth weight increased for babies born to maternal smokers and smoking fathers until the mother was 24 years old and then it plateaued. For babies born to smoking mothers and non-smoking fathers, the BW plateau occurred at a maternal age of 29 years. Among non-smoking mothers, there was a non-significant difference of -1 g in birth weight if the father was a smoker. Among smoking mothers, there was a statistically significant decrease in BW of 48 g ($p<0.01$) if the father was also a smoker, although this effect of paternal smoking abated between 1970 and 1985. The mean decrease in BW of babies with two smoking parents, adjusted for maternal age, was 201 g (95% CI 185; 218), while it was 153 g (95% CI 128; 178) if only the mother smoked. In general, the effect of maternal active smoking upon BW declined between 1970, when the mean decrease in BW was 221 g, and 1985 when the mean decrease was 178 g. From 1980 onward, there was a decrease in the effect of paternal smoking upon the BW of babies born to smoking mothers. Between 1970 and 1991, the prevalence of smoking among Norwegian men decreased from 59% to 36%; among women it declined from 32% to 27%.

The possibility of recall bias is a concern with this study as it relies on maternal memory of smoking behaviors after a period of as much as two decades. In addition, since only the presence

or absence of smoking by either parent was recorded, there is no information on exposure intensity or duration. For example, it is possible that the decrease in the apparent effect of paternal smoking was a result of the decrease in smoking prevalence among fathers during that period, consistent with an effect of paternal ETS. Thus the resulting mixing of exposure levels and durations in the analysis, and possible misclassification due to recall bias, may have obscured the effects of exposure.

Matsubara et al., 2000. This Japanese study investigates the association between smoking, both active and passive, on BW, GA, PTD, SGA, and IUGR. In Japan, pregnant women must register the pregnancy with the government. The study population included all pregnancies registered in Nagoya, Japan (n=15,207), between April 1, 1989 and March 31, 1991. At the time of registration, 15,207 women were given a self-administered questionnaire regarding smoking habits and ETS exposure; 8,624 (56.7%) women returned the questionnaire. There was no difference between women who filled out the smoking questionnaire and those who did not regarding maternal age, blood pressure, and hemoglobin. Those who filled out the questionnaire started their pre-natal care earlier than those who did not and more of them were nulliparous. Of the 8,624 women who filled out the smoking questionnaire data, 7,411 were used in the analysis. Of these, 6,335 were non-smokers, 285 (or 3.8%) were smokers, 726 (8.4%) were smokers who quit upon learning they were pregnant (mean GA at time of quitting 8.8 weeks), and 65 women were missing smoking status. Birth weights in this study were adjusted for maternal age, maternal height, body mass index, education, working status, alcohol intake, parity, infant gender, and GA at birth.

Among non-smokers, 41.5% of husbands did not smoke, 1.5% of husbands quit smoking upon learning of the pregnancy, and 56.4% of husbands smoked. When the data were stratified by paternal smoking status, there was a non-significant difference in BW between babies born to non-smokers whose husbands smoked (mean BW 3,091 g) and non-smokers whose husbands did not smoke (mean BW 3,102 g; no 95% CI or SD given). When BW was analyzed by paternal cigarettes per day (CPD), babies of non-smoking mothers exposed to ETS from 20 or more CPD were 22 g lighter (mean BW 3,104 g) than those not exposed (mean BW 3,082 g) but the difference was not statistically significant.

When stratified according to the presence or absence of ETS at work or from the husband, neonates of non-smoking ETS-exposed women were 19 g lighter than those of ETS non-exposed women (3,108 g and 3089 g, respectively; $p<0.05$). However, the data were contradictory when ETS was categorized by duration of exposure. Babies (n=1,730) born to women exposed to ETS for less than 2 hr/d were significantly lighter than in the absence of ETS (mean BW 3,082 g vs. 3,108 g; $p<0.05$), while there was no significant difference in BW when the mothers were exposed to ETS for more than 2 hr/d (mean BW 3,101 g vs. 3,108 g).

A strength of this study is the assessment of smoking early in the pregnancy, however, it's not known if there were changes in smoking behavior during the pregnancy. The authors also acknowledge that defining home ETS exposure solely by whether or not the husband smoked may have resulted in some misclassification. Of the non-smoking women whose husbands smoked, 25% reported no ETS exposure at home. This inclusion of non-exposed women in the ETS-exposed group could have led to the apparent lack of a significant ETS effects.

Hruba and Kachlik, 2000. This is a Czech study of singleton births delivered at Brno Obstetric Clinic. Medical students interviewed mothers of newly delivered babies. Little data are provided regarding the description or the selection of the cohort in this study. There were 1,097 mother-infant pairs enrolled. Of 727 never smokers, 600 were not exposed to ETS, and 127 were. Of 320 former smokers, 155 were not exposed to ETS and 165 were. There were 50 maternal smokers. The reference population were babies born to never smokers unexposed to ETS. A decrease of 64 g in mean BW was found in full-term babies born to mothers who never smoked but were exposed to ETS. While an increase in birth weight of 2 g was found in babies of former smokers exposed to ETS, former smokers not exposed to ETS had an increase of 31 g in the BW of their babies over the reference BW.

This study also examined the prevalence of LBW and PTD. Among never-smokers not exposed to ETS the prevalence of PTD was 6.5%, and 11.2% for LBW. Among never-smokers with exposure to ETS at home and work, the prevalence of either birth outcome increased to 16.7%.

The statistical significance of these data is hard to determine as there were no confidence intervals or p values reported, and no evidence of adjustment for any covariates. In addition,

there was potential reporting bias as interviewers were instructed to provide anti-smoking education.

Windham et al., 2000. This is a prospective California study of 4,099 women in a prepaid health plan who enrolled in prenatal care during the first trimester. Women were phone interviewed regarding smoking, ETS exposure, alcohol and caffeine consumption, demographics, stress, employment, and reproductive history. Outcome measures were obtained from computerized hospital records and medical charts. The model used to investigate the effect of ETS exposure was limited to non-smokers. Non-smokers (n=3646) were categorized into three groups by ETS exposure: No ETS exposure (ETS <0.5 h/d, n=2887); moderate ETS exposure (0.5-6.5 h/d, n=625, ETS); and high ETS exposure (≥ 7 h/d, n=134, ETS). Multivariate regression models of pregnancy outcomes including BW were adjusted for pre-pregnancy weight (BMI), parity, prior pregnancy losses, race, parental education, marital status, employment status, stress, caffeine and alcohol intake. In this study, there was no significant effect of ETS exposure on mean BW in non-smokers. There were 28 non-smoking pregnant women who reported 12 or more hours per day of ETS exposure, which was associated with a decrease of 88 g (SE 103) in the adjusted BW. When non-smokers were categorized by paternal smoking status, there was a decrease of 32 g in the adjusted BW (95% CI -81; 18). Data were also categorized by ethnicity and by age of the mother to investigate if ETS was associated with significant changes in birth weight in selected populations. Decreases and increases in birth weight were found in selected populations and all the 95% CIs included zero.

Among non-smokers exposed to ETS, most of the odds ratios for LBW, SGA, and PTD outcomes were elevated, but their CIs included one. Those ORs and 95% CIs are given in Tables 3.5, 3.6, 3.7. Among selected populations of non-smokers with heavy ETS exposure there were significant elevations in risk. Heavy ETS exposure in non-Caucasian, non-smoking mothers was associated with an adjusted OR for LBW of 3.8 (95% CI 1.5; 9.8). Heavy ETS exposure of non-Caucasian, non-smoking mothers was associated with an adjusted OR for PTD of 2.4 (1.1; 5.5), and for very PTD the OR was 3.8 (1.3; 10.7).

ETS exposure assessment was based on self-report of hours exposed and did not include exposure outside of the home and work. In addition, exposure was ascertained during the first

trimester and thus did not reflect any changes in exposure during pregnancy. The small number of individuals in the high exposure group limited the study's power. On the other hand, the prospective design and extensive follow-up of a population with equal access to medical care should have diminished possible confounding.

Jaakkola et al., 2001. The cohorts for this study were drawn from a larger Finnish study that enrolled all 2,751 births born into two geographically defined hospital districts between May 1996 and April 1997. Of the total number of mother-infant pairs in the original study, 1,621 self-identified as non-smokers. In the present study, 189 self identified as non-smokers with ETS exposure and 283 with no ETS. Of this latter group of non-smokers with no ETS, 142 were living with a non-smoker or a spouse who had quit over 12 months ago, and 141 lived with a smoker. Smoking status and exposure assignment were based on self-administered questionnaires, prenatal care records, birth registries, and hair nicotine. Those participating in the study provided hair for nicotine analyses. Hair nicotine levels are believed to reflect the previous two months of exposure. The final cohort assignments were based on hair nicotine levels: low nicotine exposure, 151 mother-infant pairs (hair nicotine <0.75 μg/g); medium exposure, 186 pairs (0.75 to <4.0 μg/g); and high exposure, 52 pairs (≥ 4.0 μg/g). The low nicotine group is the reference group.

The three groups based on nicotine hair levels were similar except that alcohol consumption was slightly higher in the high nicotine group (35%) than the reference group (28%). Among women who denied exposure to ETS, there was a substantial difference between those who lived with a smoker and those who did not (median 1.32 vs. 0.61 μg/g). Only 29% (N=55) of ETS exposed mothers gave quantitative data of exposure in CPD, and among these the higher the exposure the higher the hair nicotine levels (1-9 CPD; 2.68 SD ± 1.99 μg/g; 10-19 CPD, 3.4 SD ± 2.4 μg/g; ≥ 20 CPD, 5.17 SD 7.24 μg/g). Mean BW for cohorts based on hair nicotine levels were: low exposure, 3,559 g (SD ± 472); medium exposure, 3,554 g (SD ± 534); high exposure, 3,547 g (SD ± 547). Confidence intervals or p values were not given for these data. A model adjusting for confounders (infant gender, maternal age, pre-pregnancy body mass index, marital status, parental education, alcohol consumption, and employment) found a 17 g decrease in BW between the reference group and those with the highest hair nicotine levels, but the confidence interval included zero (95% CI -178; 145), and the model did not appear to control for GA. For

most of the confounders used in the model, the percents given for the reference and the high exposure groups were very similar except for increased alcohol consumption and lower education for the high-exposure groups. When hair nicotine was treated as a continuous variable in their model, there was no significant association between BW and nicotine levels (–0.91 g BW per µg nicotine per g hair, 95% CI -20; 18). Birth weight was not significantly related to ETS exposure at home (–99 g 95% CI -273; 75) or work (–101 g 95% CI -258; 56). On the other hand, preterm delivery (< 37 wks) was significantly related to ETS, particularly at hair nicotine levels above 4 µg/g, which confounds the analysis of birth weight. As maternal hair nicotine levels increased from < 4.00 to ≥ 4.00 µg/g, the adjusted ORs for PTD increased from 1.30 (95% CI 0.30; 5.58) to 6.12 (95% CI 1.31; 28.7). There was evidence of a dose-response for both exposures at home and at work. For ETS exposures, the OR for home only was 0.65 (95% CI 0.06; 6.81); work only was 2.35 (95% CI 0.50; 11.1); while the OR for both was 8.89.

Kukla et al., 2001. The European Longitudinal Study of Pregnancy and Childhood (ELSPAC) is an international longitudinal study coordinated in Great Britain. It includes approximately 40,000 women in six European countries. This study follows women during labor and delivery and their children's postnatal development. Women repeatedly filled out questionnaires, and standardized data were collected from physicians in charge. Results presented here were for 4,530 mother-infant pairs residing in the Czech Republic, of whom 2,987 were not exposed to ETS. Of the 1178 non-smokers exposed to ETS, 864 were exposed to <15 CPD and 314 were exposed to >15 CPD. There were 365 smokers of whom 298 smoked less than 10 CPD and 67 smoked more than 10 CPD. Infants born to passively and actively exposed mothers had lower mean BW, length and head circumference when compared to those with no smoke exposure. Birth weight does not appear to be corrected for GA. Compared to no ETS exposure, the babies of mothers passively exposed to <15 CPD had a mean BW that was 4 g lower, a mean length 0.01 cm longer, and a mean head circumference that was higher by 0.11 cm; none of which was statistically significant. The babies of mothers passively exposed to >15 CPD had a mean BW that was 49 g ($p<0.06$) lighter, a mean length 0.34 cm ($p<0.01$) shorter, and a mean head circumference of 0.01 cm larger. By comparison, babies of mothers smoking <10 CPD had a BW 79 g ($p<0.01$) lighter, they were 0.48 cm ($p<0.001$) shorter, and a head circumference that was smaller by 0.28 cm ($p<0.001$). The data indicate that high maternal ETS exposure affects

fetal growth, specifically BW and length. The data would be more compelling if growth parameters had been adjusted for GA and other predictors of pregnancy outcome instead of a statement that they were similar. Occupational ETS exposure was not ascertained. As a result some women included as non-smokers may have been exposed at work thus diminishing a possible ETS effect.

Kharrazi et al., 2001. This is a prospective Californian study of 2,777 mother-infant pairs and 19 mothers whose fetuses died after 20 weeks gestation. Cotinine levels in maternal serum were determined between the 15th and 19th week of gestation using isotope-dilution high performance liquid chromatographic/atmospheric pressure ionization tandem mass spectrometry. This assay method is the best method currently available with a limit of detection (LOD) for cotinine of 0.05 ng/ml. Only mothers with cotinine levels <10 ng/ml were included in the study. Approximately 30% of mothers had a cotinine level below the LOD, while about 5% of mothers had cotinine levels between 1 and 10 ng/ml. It is possible that the group with the levels above 1 ng/ml included light active smokers especially if the levels were above 3 ng/ml. Otherwise, it is probable that the cotinine levels reported here reflect passive smoking. Indeed, if there were light active smokers in the unexposed group, the strength of the effect of ETS would be underestimated. Five co-variates were used in the linear and logistic regression models: maternal ethnicity, maternal age, parity, source of prenatal care insurance, and infant gender. Models investigating BW controlled for GA.

There was an inverse dose dependent decrement in BW with maternal cotinine levels. Infants were segregated by maternal cotinine levels into four groups - <0.05 ng/ml (LOD, reference group), 0.05-<0.1 ng/ml (Group A), 0.1-0.5 ng/ml (Group B), and 0.5-10.0 ng/ml (Group C). There was a 20 gram difference in mean BW between those in the reference group (BW 3444 g) and Group A (BW 3424 g). There was a 39 g difference between the reference group and Group B (BW 3383 g). There was a 111 g difference between the reference group and Group C (BW 3333 g). In a linear regression model which included the co-variates, there was approximately a 30 g decrease in the mean BW in babies whose mothers had cotinine levels between 0.1 and 1.0 ng/ml, when compared to those with cotinine levels below the LOD. While for infants whose mothers' cotinine levels were above 1 ng, the decrease was 100 g. The decline in BW per log unit of cotinine was significant (p=0.04).

The population attributable risk of adverse pregnancy outcomes was calculated for exposure to ETS above 0.05-ng/ml cotinine. The unadjusted OR for an adverse pregnancy outcome (fetal demise, PTD, LBW) was 1.47 (95% CI 1.19; 1.83) and the adjusted OR was 1.36 (95% CI 1.07; 1.72) (Table 3.5). The subsequent ORs are for each unit change in the log of the cotinine concentration. The unadjusted OR for PTD was 1.38 (95% CI 1.06; 1.81) and the adjusted OR was 1.29 (95% CI 0.97-1.72). The OR for PTD comparing the 20% with the highest ETS exposure to those whose cotinine levels that were ≤ 0.05 ng/ml was 1.78 (1.01; 3.13) (Kharrazi, pers comm.). The unadjusted OR for a term LBW baby was 1.63 (95% CI 1.10; 2.41) and the adjusted OR was 1.42 (95% CI 0.91; 2.21). This is an excellent study in that it was able to detect differences in outcomes between levels of ETS exposure as defined by the biomarker. The study was able to detect differences in outcome among groups that had cotinine levels below 1.0 ng/ml.

Dejmek et al., 2002.. This is a retrospective study of 6,866 mother-infant pairs conducted in the Czech Republic. Data regarding smoking habits and ETS exposure before and during each trimester of pregnancy were obtained by questionnaire during the hospitalization for birth and by medical record review. The analysis controlled for maternal age, geographic location of home, ethnicity, parental education, and parity, sex of infant, maternal height, pre-pregnancy weight, and alcohol consumption and season of the year. There were 4,309 women who were non-smokers prior to conception, 1,500 were moderate smokers (1-10 CPD), and 1,049 were heavy smokers (>10 CPD). ETS exposure was defined as exposure to smoke from five or more CPD, smoked by another person in the presence of the mother. Among non-smokers 25% were ETS exposed (mean ETS 11 CPD), while 67% of moderate smokers were ETS exposed (mean ETS 14 CPD) and 85% of heavy smokers were ETS exposed (mean ETS 23 CPD). Among those smoking prior to pregnancy, 734 quit during the first trimester, 467 quit during the second trimester, and 52 quit during the third trimester.

The adjusted decrease in BW for non-smokers exposed to ETS from 5 or more CPD was 53 g (95% CI 24; 82). The adjusted OR for a LBW baby if the mother was a non-smoker exposed to ETS was 1.51 (95% CI 1.02; 2.26) (from Table 2 in Dejmek *et al.*,2002). The adjusted OR for IUGR among non-smokers exposed to ETS was 1.08 (95% CI 0.82; 1.43). A strength of this

study was the collection of smoke exposure data at several points during the pregnancy so that the analysis reflected changes in ETS exposure as smoking habits changed.

3.2.2. Animal Studies of ETS and BW, IUGR

Animal studies reporting the effects of maternal ETS exposure during pregnancy on fetal and birth weights are limited in number. In a study by Ji *et al.* (1998), pregnant rats were exposed to aged and diluted sidestream smoke for 6 hr/d, 7 d/wk starting on gestation day 5. While smoke exposure was seen to alter specific protein expression in fetal lung, the weights of fetuses collected at gestational days 14, 18 and 21 were not significantly different between exposed and control animals. In contrast to these results, Nelson *et al. (*1999b) found that BWs in rats were decreased by 41% compared to unexposed controls following exposure of the pregnant dam to sidestream smoke from one cigarette per day for one week if the exposure occurred during the first week of pregnancy. The same exposure starting in the third week of pregnancy resulted in a 73% reduction in BW. A significant dose-dependent decrease in intrauterine growth and BW with smoke exposure was observed after exposure to 0-3 cigarettes per day ($p < 0.001$). The reasons for the discrepancy between these studies in BW data are not clear but are likely related, in part, to different exposure conditions. The exposure conditions are not well characterized in the study by Nelson *et al.* thus limiting comparison with the study by Ji *et al.*

3.2.3. Discussion of Fetal growth and preterm delivery

In this update, 18 studies were reviewed that investigate the relationship between maternal ETS exposure and fetal growth as measured by BW or the incidence of an adverse fetal growth outcome (LBW, SGA or PTD). These studies represent several geographically separated areas (North America, South America, Europe and Asia). Most studies done in the past decade controlled for confounders known to be associated with decreased fetal growth.

3.2.3.1. Birth weight data

There are numerous studies from the previous and current reviews that provide strong evidence for an association between ETS exposure and decrements in BW. The following conclusion appeared in the prevous review.

> *There appears to be sufficient evidence that ETS is associated with a decrement in birthweight (and fetal retardation), based on all sources of data with primary emphasis on the high quality epidemiological studies. The effect is of a small magnitude (perhaps 25-50 grams) that may not be clinically significant for an individual infant at low risk. Yet, if the entire birthweight distribution is shifted lower with ETS exposure, as it appears to be with active smoking, infants who are already compromised may be pushed into even higher risk categories.*

Those studies combined with the more recent ones indicate ETS exposure is associated with a decrease in BW (in the non-smoking mother) in the range of 10-100 g. This includes evidence of a dose-response down to very low levels of exposure (Kharrazi *et al.*, 2001). Studies from both the previous and current documents that reported BW data with statistics are shown in Figure 3.1 in chronological order. Table 3.3 summarizes the eight studies that reported BW data and included maternal smokers in their ETS model. A decrease in BW was associated with ETS exposure in all eight studies although one (Ahluwalia *et al.*, 1997) reported a non-significant increase in BW for infants of mothers under 30 years of age. The BW decrements ranged from 4 to 100 g, and the results were statistically significant in three studies. Four studies (Jedrychowski and Flak, 1996; Horta *et al.*, 1997; Wang *et al.*, 1997; Hruba *et al.*, 2000) considered GA in their analyses. For the studies that controlled for GA, the BW decrements were 30 to 64 g. Other studies had larger decreases in BW, some of which were similar to those reported for active smokers. However, the inclusion of active smokers in the model and the lack of control for GA undermine the reliability of the magnitude of BW decrements reported by these studies.

Table 3.3 ETS and BW; Studies that Included Maternal Smokers

Reference	Total	MNS[1] no ETS	MNS[1] w/ ETS	Change in BW (g) (95% CI)	Confounder, Covariate Adjustments[2]
Jedrychowski & Flak 1996	1219	246	532	ETS=10 CPD, -57.9g p = 0.004	GA, Sex, Par, Oth
Ahluwalia *et al.*, 1997	17412	10639	2855	<30 yo +8.8 g (-26; +44) >30 yo -90.0 g (-181; +1)	Eth, Par, Alc, MWt, Oth
Horta *et al.*, 1997	5166			-30g; p<0.053	GA, MA, Eth, Par, SES, MWt, MHt, Oth
Lodrup Carlsen *et al.*, 1997	803	483	96	–100g; p=0.043	None reported
Wang *et al.*, 1997	740	403	80	data suggestive of ETS effect on BW	GA, Eth, Par, Alc, MWt, MHt, Oth
Hruba & Kachlik 2000	1097	755	292	-64 g; no statistics given	GA
Kukla *et al.*, 2001	4530	2987	1378	ETS < 15 CPD –4g; n.s. ETS > 15 CPD –74g; p<0.063	None reported
Dejmek *et al.*, 2002	6866	3710	1797	-41 g (-5, -77)[3]	Sex, Eth, Par, Alc, SES, MWt, MHt, Oth

[1] MNS: maternal non-smoker (Blank – number not given); [2] Alc: alcohol use; Eth: ethnicity; GA: gestational age; MHt: maternal height; MWt: maternal weight; Oth: other; Par: parity; SES: socioeconomic status; Sex: sex of newborn. [3] Statistically significant change.

Studies that excluded maternal smoking from their analysis of the association between BW and ETS exposure are summarized in Table 3.4. Six of the eight studies took GA into account. One study (Windham *et al.*, 2000) found an increase in BW of 8 g, otherwise all studies found a decrease or no difference in BW. Of these, one study reported a statistically significant decrement in BW. This study by Kharrazi *et al.* (2001) was prospective and used cotinine to quantitate exposure to ETS. The reference cohort had plasma cotinine levels below 0.01 ng/ml. There were three cohorts above 0.01 ng/ml cotinine. The smallest cohort had the highest levels (1 - 10 ng/ml) and may have included light active smokers, but the levels of the other two cohorts (0.01-0.1 ng/ml and 0.1-1 ng/ml) are consistent with ETS exposure. There was a 20 to 40 g decrease in BW associated with maternal plasma cotinine levels between 0.01 and 1 ng/ml. This is similar to the difference in BW reported by Haddow *et al.* (1988) between those with plasma cotinine levels in the lowest group (<0.5 ng/ml) compared with those with cotinine levels between 1.1 and 9.9 ng/ml. Both Haddow and Kharrazi had similar magnitudes in the BW decrements between those with the lowest cotinine levels and those with the highest (104 g and

111 g, respectively). The study by Martinez *et al.* (1994) found a similar magnitude of BW decrement (34 g) associated with paternal smoking when compared to the Kharrazi study.

Included in the studies summarized above are two meta-analyses addressing the effects of ETS on BW. The pooled estimates of decrements in BW were statistically significant and similar between the studies: -24.0 g (95% CI –39.3; -8.6)(Windham *et al.*, 1999) and –31 g (95% CI –44; -19) (Peacock *et al.*, 1998).

Table 3.4 ETS and BW; Studies that Excluded Maternal Active Smokers

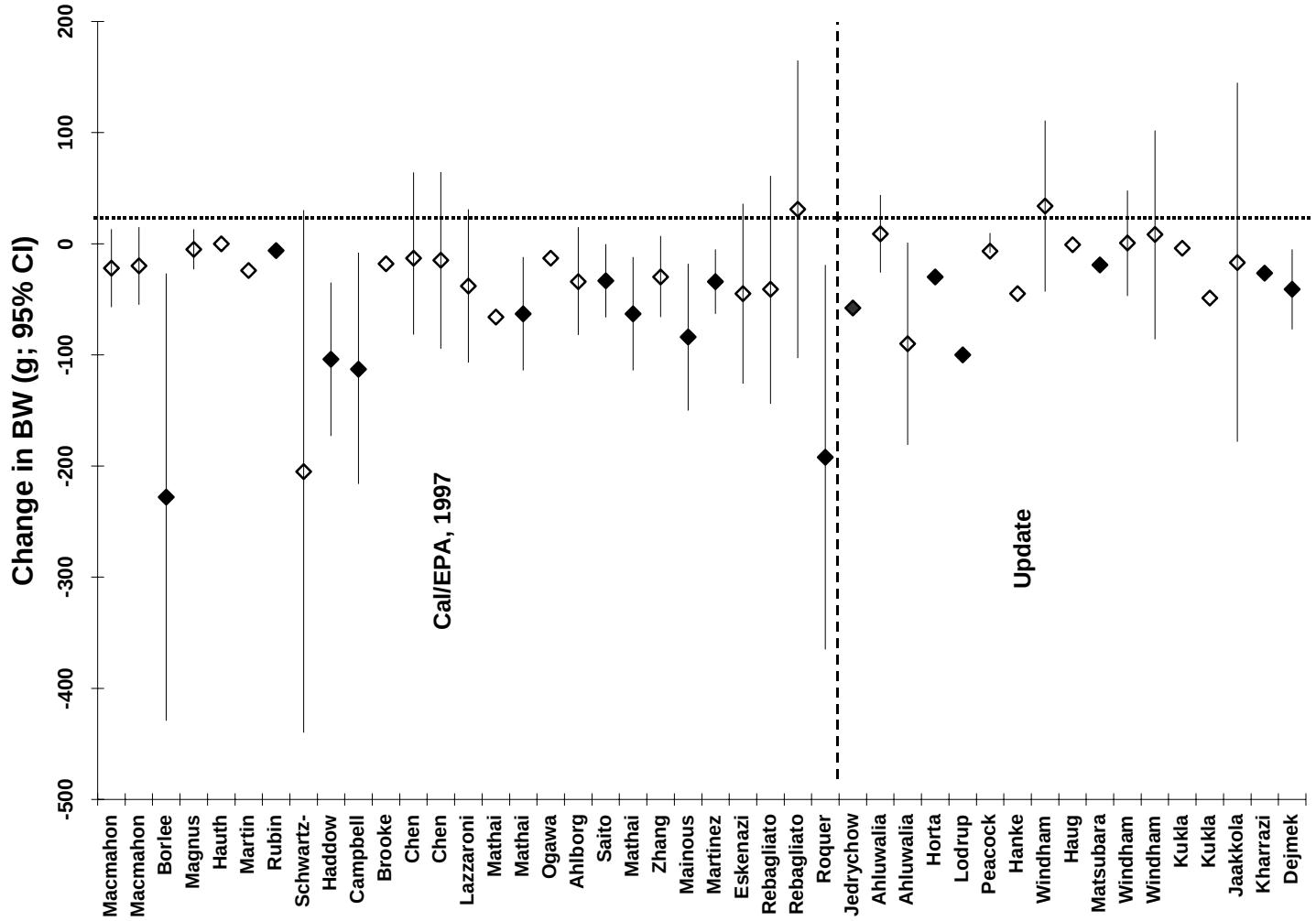
Reference	Total N	MNS[1] no ETS	MNS w/ ETS	Change in BW (95% CI)	Confounder, Covariate Adjustments[2]
Peacock *et al.*, 1998	703	420	283	-6.7 g (–8.4; +9.7)	GA, Sex, Par, MHt
Hanke *et al.*, 1999	1751	924	827	n.s.	GA, MHt, Oth
Windham *et al.*, 1999	992			+34 g (-43; +111)	GA, Eth, Alc, Oth
Haug *et al.*, 2000	22883			-1 g; n.s.	
Matsubara *et al.*, 2000	8624	2693	3586	n.s.	GA, Sex, Par, Alc, MWt, MHt
Windham *et al.*, 2000	4099	2887	759	low ETS +0.68 g (-47; +48) high ETS +8.2 g (-86; +102)	GA, MA, Eth, Par, Alc, SES, MWt, MHt
Jaakkola *et al.*, 2001	477	288	233	-17 g (–178; +145)	
Kharrazi *et al.*, 2001	2796	951	1845	-20 to -111 grams; p = 0.04	GA, Sex, Eth, SES, Oth

[1] MNS: maternal non-smoker; [2] Abbreviations: Alc: alcohol use; Eth: ethnicity; GA: gestational age; MHt: maternal height; MWt: maternal weight; Oth: other; Par: parity; SES: socioeconomic status; Sex: sex of the newborn.

In this update, there is a consistent finding of a decrease in BW associated with maternal ETS exposure that was substantiated in one of two animal studies. These findings are in the same range as that reported in the previous document (25-50 g) and lend further support for the previous suggestion of a causal association. Most of these studies considered pertinent confounders, as well as GA, in their analysis. One study was able to validate the ETS exposure and BW decrements with maternal plasma cotinine levels below 1 ng/ml. This magnitude of BW deficit may not seem clinically significant, but this is a mean deficit. As with fetuses of smokers (Wang *et al.*, 2002), some fetuses of maternal non-smokers with ETS exposure may be at greater risk than others based on genetic make up. Future studies may be able to elucidate this.

Figure 3.1 The Effects of ETS on Birth Weight.

The mean change in BW with maternal ETS exposure from studies reported in the previous document (Cal/EPA, 1997) and those included in this update. Statistically significant values are represented by solid diamonds; statistically non-significant values by open diamonds.



3.2.3.2. Adverse Fetal Growth Outcomes

There are 25 studies that investigated the association between maternal ETS exposure and an adverse fetal growth outcome (LBW, SGA, IUGR and PTD), ten of which were published since the previous document. Table 3.A (Appendix) presents data from all of the studies that reported outcomes for LBW, SGA, IUGR, and PTD. Pre-term delivered newborns are not necessarily fetal growth retarded, but PTD is included here because many pre-term delivered babies have a BW below 2500 g, the definition of LBW. Additionally, PTD, LBW, IUGR, and SGA are commonly studied together. Of the ten new studies, five excluded active smokers and three analyzed non-smokers exposed to ETS as a separate stratum. Six studies found an increased risk of an adverse fetal growth outcome while three found no increased risk (OR or RR is $\leq$1.0), or only reported the results as non-significant.

3.2.3.3. Low Birth Weight

In the previous document it was suggested that the studies supported a slight increase in risk for LBW in association with ETS. However, due to wide confidence intervals, the results were also consistent with no effect. The more recent studies provide evidence that strengthens this association. Included in this update are seven studies reporting LBW data. Six found an increased risk of LBW associated with ETS exposure with ORs ranging from 1.18 to 3.8 (Table 3.5), two of which were statistically significant. The study by Ahluwalia *et al.* (1997) is a large prospective study. ETS exposure was not associated with an increased risk for LBW among maternal non-smokers under the age of 30 years, but it did increase the risk of LBW if the mother was 30 years or older. This is consistent with studies of smokers that have found that the more years a woman has smoked, the greater the BW deficit. It is postulated that this is due to the accumulation of toxic heavy metals over the years of smoking (Kuhnert, 1988). Cigarette smoke contains lead and cadmium and their elimination half-lives are measured in years. Smoking is the major determinant of plasma cadmium levels even among those residing adjacent to cadmium smelters (Lagerkvist *et al.,* 1993). Maternal non-smokers with ETS exposure, over the age of 30, may have been exposed to ETS and accumulating cadmium for years (Dempsey and Benowitz, 2001; Kuhnert *et al.*, 1988).

Table 3.5 ETS and LBW

Reference	Total N	MNS[1] no ETS	MNS[1] w/ETS	LBW OR, RR (95% CI)	Confounder, Covariate Adjustments[2]
Jedrychowski & Flak 1996	1115	452	512	1.46 (0.83; 2.6)	GA, Sex, Par
Ahuwalia *et al.*, 1997	17412	10639	2855	0.97 (0.76; 1.23) < 30yo 2.4 (1.5; 3.9) ≥ 30yo3	Eth, Par, Alc,MWt, Oth
Horta *et al.*, 1997	5166			1.18 (0.94; 1.48)	GA, MA, Eth, Par, SES, MWt, MHt, Oth
Windham *et al.*, 2000	4099	2887	759	1.8 (0.82; 4.1) high ETS 3.8 (1.5;9.8) " , non-caucasian	GA, MA, Eth, Par, Alc, SES, MWt, MHt, ExAS
Jaakkola *et al.*, 2001	477	288	233	n.s.	ExAS
Kharrazi *et al.*, 2001	2796	951	1845	Adverse Outcome 1.36 (1.06;1.72)[3] LBW: 1.42 (0.91; 2.21)	Sex, Eth, SES, Oth, ExAS
Dejmek *et al.*, 2002	6866	3710	1797	1.51 (1.02; 2.26)[3]	Sex, Eth, Par, Alc, SES, MWt, MHt, Oth

[1] MNS: maternal non-smoker (Blank – number not given); [2] Abbreviations. Alc: alcohol use; Eth: ethnicity; ExAS: excludes active smokers; GA: gestational age; MHt: maternal height; MWt: maternal weight; Oth: other; Par: parity; SES: socioeconomic status; Sex: sex of newborn. [3] Statistically significant change.

The study by Dejmek *et al.* (2002) is a well-designed study. Smoking histories and ETS exposures were obtained during hospitalization for the birth and numerous covariates and confounders were included in the ETS model. The adjusted OR for LBW associated with ETS exposure among maternal non-smokers was 1.51 (95% CI 1.02; 2.26). This is very similar to the risks reported by other studies given in Table 3.5. The OR for LBW associated with heavy smoking was 2.31 (95% CI 1.34; 4.08).

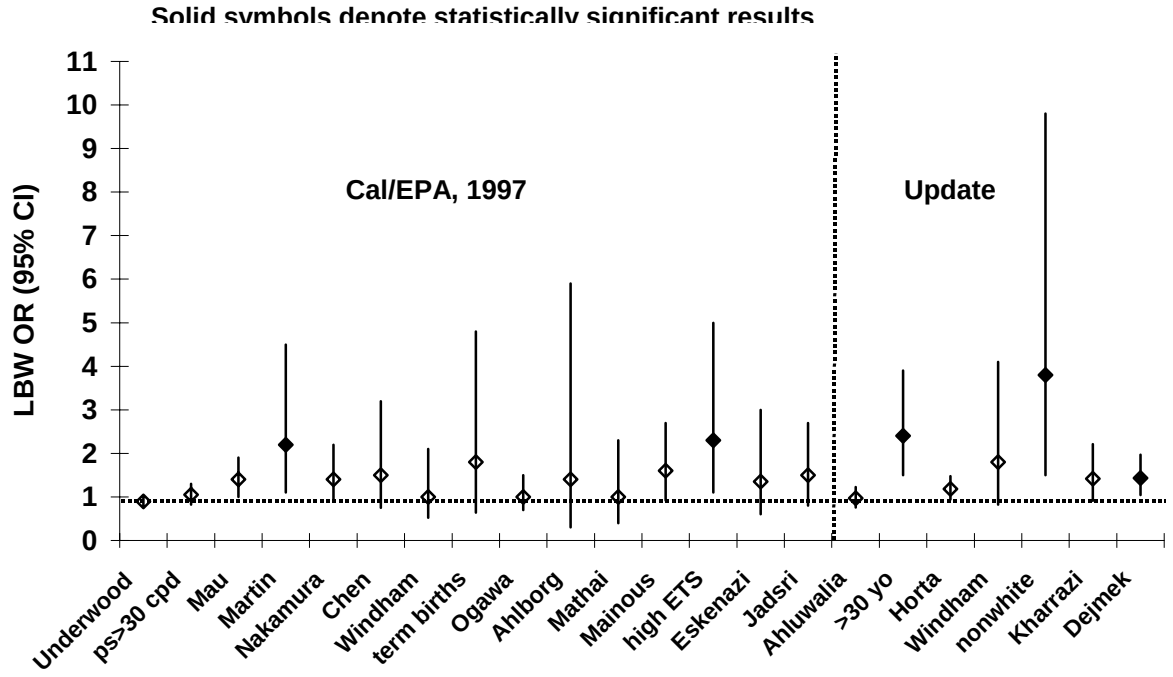
The study by Windham *et al.* (2000) limited their enrollment to maternal non-smokers and stratified their data by ethnicity. They found a significant increase in the adjusted OR for LBW among non-Caucasian women. This is consistent with studies of maternal smokers that have found higher ORs for LBW, SGA and pre-term delivery among African-American smokers compared to Caucasians.

The study by Kharrazi *et al.* (2001) is also a prospective study limited to maternal non-smokers that showed an increased risk of an adverse pregnancy outcome (LBW, SGA or PTD) associated with ETS exposure. They did not find a statistically significant increase in LBW but their OR of 1.42 is similar to the larger study by Dejmek *et al.* (2002). The Kharrazi study is important because the ETS exposure was defined by maternal cotinine levels. Their assay method is state

of the art and the lower limit of detection is well below all other published studies. The levels of cotinine for two of the three ETS exposure groups and the reference group were below 1 ng cotinine/ml plasma. It is unlikely that active smokers were among those whose levels are below 1 ng/ml.

Since the previous monograph there have been three studies that found a statistically significantly elevated risk of delivering an LBW baby associated with ETS exposure among women who were non-smokers during their pregnancies. These data indicate that ETS exposure is associated with an increased risk of delivering a LBW baby.

Figure 3.2 ETS and Risk of Low Birth Weight



3.2.3.4. SGA, SFD and IUGR

Fetal growth retardation (SGA, SFD, IUGR) is intrinsically difficult to study compared to LBW, which is an easy outcome to document, or PTD, which is a relatively easy outcome to determine. An accurate measure of GA is required for SGA, SFD and IUGR because these measures are gestationally dependent. Late entry into prenatal care or poor prenatal care, which are associated with SGA, SFD and IUGR make it difficult to accurately estimate GA. Fetal or pregnancy conditions that result in PTD are often associated with poor fetal growth.

With respect to studies of ETS and IUGR, the previous document suggested that taken together "*they support a slight increase in* [risk of] *LBW or IUGR in association with ETS exposure.*" This association has been strengthened in this update. Seven studies have reported data regarding the adverse fetal growth outcomes of SGA, SFD, and IUGR. One study found a reduced risk of adverse growth outcome, four studies found no increase in the risk, while two studies found a statistically significant increased risk. The increased risks ranged from 1.19 to 3.9 (Table 3.6). Of the studies finding statistically significant increases in risk of SGA, SFD or IUGR associated with maternal ETS exposure, one is Brazilian (Horta *et al.*, 1997), and the other is Swedish (Dejin-Karlsson *et al.*, 1998).

Table 3.6 ETS and SGA, SFD, IUGR

Reference	Total N	MNS[1] no ETS	MNS[1] /ETS	IUGR, SGA, SFD OR, RR (95% CI)	Confounder, Covariate Adjustments[2]
Ahluwalia *et al.*, 1997	17412	10639	2855	SGA 0.97 (0.8; 1.3) <30yo 1.3 (0.8; 2.2) ≥30yo	Eth, Par, Alc, MWt, Oth
Horta *et al.*, 1997	5166			IUGR 1.33 (1.05; 1.68)[3]	GA, MA, Eth, Par, SES, MWt, MHt, Oth
Dejin-Karlsson *et al.*, 1998	854	247	345	SGA 3.9 (1.4; 10.7)[3]	GA, MA, Eth, Par, Alc, Drg, SES, MWt, MHt, Oth
Nafstad *et al.*, 1998	163	68	54	SGA 1.0 (0.4; 2.1)	GA, Sex, MWt, MHt, Oth
Windham *et al.*, 1999	992			SGA 1.4 (0.79; 2.5)	GA, Eth, Alc, Oth, ExAS
Matsubara *et al.*, 2000	7411			IUGR 0.95 (0.72; 1.26)	Sex, MA, Par, Ed, Alc, MHt, MWt,
Dejmek *et al.*, 2002	6866	3710	1797	IUGR 1.08 (0.82; 1.43)	Sex, Eth, Par, Alc, SES, MWt, MHt, Oth

[1] MNS: maternal non-smoker (Blank – number not given); [2] Abbreviations. Alc: alcohol use; Ed: maternal education; Eth: ethnicity; ExAS: excludes active smokers; GA: gestational age; MHt: maternal height; MWt: maternal weight; Oth: other; Par: parity; SES: socioeconomic status; Sex: sex of newborn. [3] Statistically significant change.

The study by Horta *et al.* (1997) carefully investigated IUGR, LBW, and GA. This study comprised 80% of all births in one town for one year. Smoking histories were taken by study personnel during the postpartum hospital stay and newborns were prospectively examined for GA by trained study personnel using the Dubowitz method (the most widely used examination instrument to determine GA of newborns). Babies were sorted into four categories based on BW and GA: BW ≤ 2500 g and GA <37 weeks; BW ≥ 2500 g and GA ≥ 37 weeks; BW ≥ 2500 g and GA < 37 weeks; and BW ≤ 2500 g and GA > 37 weeks. Newborns were also evaluated for

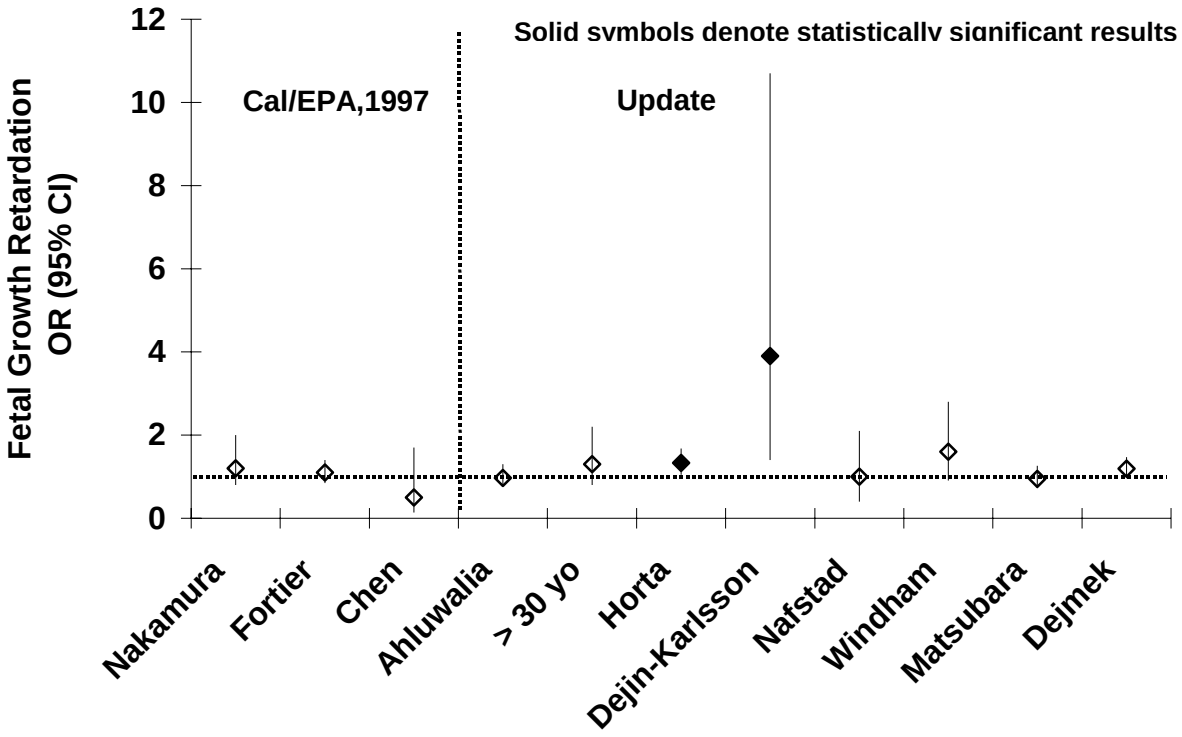
growth retardation. This was a thorough postpartum evaluation of growth, and the study controlled for most of the relevant confounders or covariates. They found an adjusted OR for SGA of 2.0 (95% CI 1.5; 2.69) for light active smokers (1-5 CPD). It was 2.48 (95% CI 1.68; 3.68) for heavy active smokers (≥ 20 CPD) and, after controlling for maternal smoking, the OR for SGA associated with paternal smoking was 1.33 (95% CI 1.05; 1.68).

The Swedish study by Dejin-Karlsson *et al.* (1998) also reported a significant increase in risk for SGA associated with ETS exposure (adjusted OR 3.9; 95% CI 1.4; 10.7); while that for smokers was 6.0 (95% CI 2.1; 17.5). Although this odds ratio is about double that reported by other studies, this is a very well designed prospective study that controlled for most of the relevant covariates and confounders. The study population was 87.7% of all nulliparous mothers who delivered in one town during one-year interval. Gestational age was confirmed by sonographic exam at 20 weeks gestation. Growth curves were based on Swedish and Danish ultrasonographic data.

Both of these are thorough studies in which fetal growth was the primary outcome of interest. In one study, all newborns had a Dubowitz exam by a trained examiner to determine GA and fetal growth retardation. In the other study, GA was confirmed using sonography and newborns that weighed 2.5 standard deviations below the age-related means were classified as SGA. These studies strongly indicate that there is an increased risk to fetal growth retardation associated with maternal ETS exposure.

Evidence for significant fetal growth restriction was also observed by Nelson *et al.* (1999) in rats after exposure to sidestream smoke from 1, 2 or 3 cigarettes per day.

Figure 3.3 ETS and Risk of Fetal Growth Retardation (IUGR, SGA)



3.2.3.5. Pre-Term Delivery (PTD)

On the basis of five studies reporting data on PTD, two prospective, two retrospective, and one of uncertain type, the previous document concluded that there was little evidence of an association between ETS and PTD. In this update, there are four new studies that reported data regarding PTD (Table 3.7). In contrast to the previous document, these studies all reported an increased risk of PTD associated with ETS exposure with OR or RR ranging from 1.29 to 3.8, three of which were statistically significant.

Table 3.7 ETS and PTD

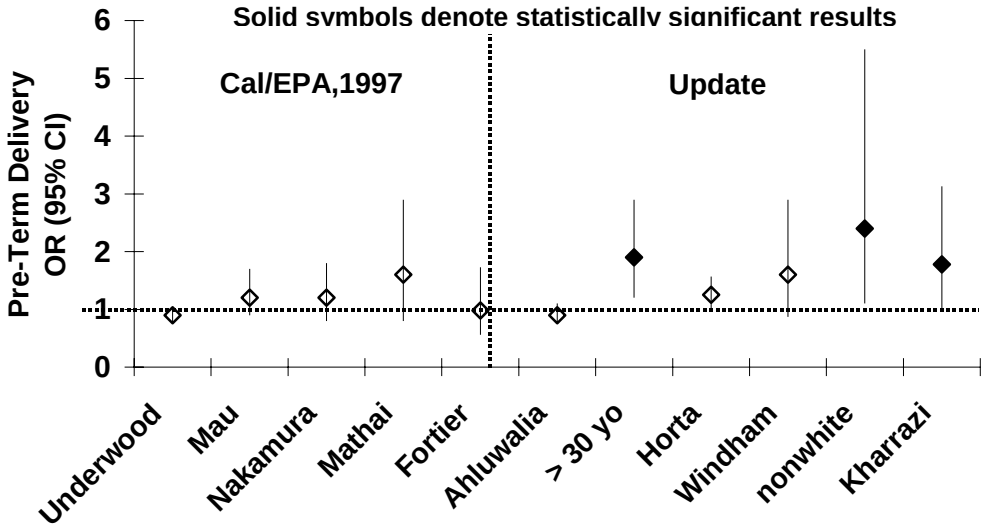
Reference	Total	MNS[1] no ETS	MNS[1] w /ETS	PTD OR, RR (95% CI)	Confounder, Covariate Adjustments[2]
Ahluwalia *et al.*, 1997	17412	10639	2855	0.9 (0.8; 1.1) < 30 yo 1.9 (1.2; 2.9) ≥ 30 yo[3]	Eth, Par, Alc, MWt, Oth
Horta *et al.*, 1997	5166			1.25 (0.99; 1.57)	GA, MA, Eth, Par, SES, MWt, MHt, Oth
Windham *et al.*, 2000	4099	2887	759	1.6 (0.87; 2.9) high ETS 2.4 (1.0; 5.3) "very preterm, Ethnicity not Caucasian" 2.4 (1.1; 5.5) high ETS[3] (3.8; 10.7) "very preterm"[3] 2.8 (1.2; 6.6) > 30 yr [3]	GA, MA, Eth, Par, Alc, SES, MWt, MHt, ExAS
Kharrazi *et al.*, 2001	2796	951	1845	Adverse Outcome 1.36 (1.06;1.72)[3] PTD: 1.78 (1.01; 3.13)	Sex, Eth, SES, Oth, ExAS

[1]MNS: maternal non-smoker (Blank – number not given); [2]Abbreviations. Alc: alcohol use; Eth: ethnicity; ExAS: excludes active smokers; GA: gestational age; MHt: maternal height; MWt: maternal weight; Oth: other; Par: parity; SES: socioeconomic status; Sex: sex of newborn. [3] Statistically significant change

The study by Windham *et al.* (2000) stratified subjects by age and found that among all non-smoking women, there was no significant risk of PTD or very PTD with low exposure to ETS. But among women 30 years and older, there was a significant risk of PTD (adjusted OR 2.8; 95% CI 1.2; 6.6) associated with ETS exposure. This increased risk of PTD associated with older women (>30 years) was also previously seen by Ahluwalia *et al.* (1997) in a very large study (n=17,412) in which the adjusted OR for PTD for women 30 years and older was 1.9 (95% CI 1.2; 2.9). Windham *et al.* (2000) stratified subjects by ethnicity and found increased risk of PTD among non-Caucasians with high ETS exposure; the adjusted OR for PTD was 2.4 (95% CI 1.1; 5.5) while for very PTD it was 3.8 (95% CI 1.3; 10.7).

One of the prospective studies reporting a significant risk for PTD used a state of the art assay method to determine cotinine levels, and two of the three ETS exposure groups had second trimester maternal plasma cotinine levels below 1 ng/ml (Kharrazi *et al.*, 2001). This study found a significant increase in PTD for the top 20% of ETS-exposed mothers compared to those whose cotinine levels were below the level of detection. The retrospective study by Horta *et al.* (1997) found an elevated risk although it did not reach statistical significance (adjusted OR 1.25; 95% CI 0.99; 1.57). These four studies taken together provide evidence supportive of a causal association of maternal ETS exposure with PTD.

Figure 3.4 ETS and Risk of Preterm Delivery



3.3. Spontaneous Abortion (SAB) and Perinatal Death

Perinatal death encompasses a wide variety of causes or diagnoses (*e.g.*, *abruptio placenta*, premature rupture of membranes, severe malformation), which may result from different etiologic factors. Identification of confounders is particularly complex. As prematurity and LBW are risk factors for neonatal death, BW and GA should be considered when studying perinatal mortality. When examining spontaneous abortion, maternal age, prior history of pregnancy loss and socioeconomic status indicators, at a minimum, should be considered as potential confounders.

Chatenoud et al., 1998. This is an Italian case-control study investigating parental smoking habits before and during the first trimester of pregnancy in 359 cases of spontaneous abortion (GA ≤ 12 weeks) and 685 control cases of term deliveries (GA > 37 weeks). Smoking behavior of the mother and father was based on maternal recall during interviews. Confounders included in the multiple logistic regression were: hospital, maternal age, education, marital status, maternal family history of SAB and miscarriages, and alcohol and coffee habits during the first trimester. The OR for SAB associated with parental smoking was 0.8 (95% CI 0.7; 1.0). However, maternal smokers and non-smokers were included in the analyses of the effect of paternal smoking and no adjustment for maternal active smoking was indicated. The inclusion

of maternal smokers and non-smokers in the control group makes the significance of the risk calculation hard to interpret.

Windham et al., 1999. This is a prospective study of over 5,000 pregnancies conducted in California. Women in a prepaid health plan who sought prenatal care during the first trimester were phone interviewed within two weeks of their first prenatal care visit, and their smoking habits and ETS exposure were obtained. Birth outcomes were obtained from computerized hospital records and medical charts. There were 499 SABs, 4,613 live births, and 32 stillbirths (outcomes for 198 could not be determined). The ETS exposure was ascertained during interview as number of hours per day spent in the presence of smokers, and only examined among women who were non-smokers (N=4,209) of whom 1,178 were ETS exposed. The adjusted odds ratio (OR) for SAB among ETS exposed non-smokers was 1.01 (95% CI 0.8; 1.27). The risk of SAB among ETS exposed non-smokers was increased if there was moderate alcohol consumption or heavy caffeine consumption, although it was statistically significant only for caffeine consumption greater than 300 mg/d. They also found an increase in the odds ratio for SAB among active smokers, although the 95% confidence intervals included one.

As with other studies that rely exclusively on self-report for ETS exposure, there may have been some misclassification bias. Also, the amount of spousal smoking during pregnancy was not quantified. Thus any efforts by the parents to quit smoking, or prevent ETS exposure prior to conception or upon learning of the pregnancy, could have limited exposure to ETS. This would result in an under-estimation of the risk of ETS exposure.

3.3.1. Discussion: ETS and Spontaneous Abortion and Perinatal Mortality

The following definition and conclusions are from the previous monograph (Windham, 1999) and remain unchanged by more recent studies:

> *For the purposes of this discussion, perinatal mortality is defined as death in the period from 20 weeks gestation to 28 days post-delivery. Perinatal mortality includes stillbirths (fetal death from 20 weeks to term) and neonatal deaths (death between birth and 28 days of life). Relatively few studies have assessed the effect of ETS exposure on perinatal mortality. Spontaneous abortion or miscarriage is currently defined as pregnancy loss in*

the first 20 weeks of gestation, but was defined as loss up to 28 weeks in older reports. Some authors have combined spontaneous abortions with stillbirths to look at prenatal and perinatal deaths.

In conclusion, there is some epidemiological evidence that ETS exposure may play a role in the etiology of spontaneous abortion, which is consistent with some but not all studies of active smoking. More work is needed because of the few studies available and inconsistent findings.

Two studies have been published since the previous monograph investigating the association between ETS exposure and pregnancy wastage (Windham *et al.*, 1999; Chatenoud *et al.*, 1998) and neither found a significant association. While paternal smoking may result in maternal ETS exposure, it may also affect sperm so that any association between paternal smoking and fetal wastage may be unrelated to ETS exposure. Overall, the limited data do not support an increase in risk of pregnancy wastage associated with maternal ETS exposure, but gene-environment interactions may affect the risk of pregnancy wastage. One Dutch study of women with recurrent early pregnancy losses found that the frequency of one glutathione-S transferases gene (GSTP1-1b1b alleles) was significantly higher among women with recurrent pregnancy losses compared to controls (Zusterzeel *et al.,* 2000). Glutathione transferases are involved in the metabolic elimination of some cigarette toxins. Based on genetic susceptibility, it may be that some pregnancies are more vulnerable to maternal ETS exposure than others. Future research in this direction may help clarify this issue.

3.4. Human Studies of ETS and Congenital Malformations

Congenital malformations (specifically structural) include a wide variety of diagnoses, such as neural tube defects (*e.g.*, anencephaly, spina bifida), cleft palate, and defects of the genitourinary and the cardiovascular systems, among others. About 3 to 10 percent of births are affected depending upon the definition and method of detection. Some studies limit cases to major malformations, whereas others use a broader definition of anomaly. There is some controversy about how to categorize diagnoses, *e.g.*, by organ system or embryologic origin. The same malformation may be associated with different etiologies. Potential confounding variables are

not well defined, but maternal age, prior reproductive history, socio-economic status, and nutritional intake should be considered.

The literature on the relationship of active maternal smoking to congenital malformations is inconsistent, and the 2001 Surgeon General's report found no association between congenital malformations and active smoking (U.S.DHHS, 2001). More recent research in the area of congenital malformations has focused on genetic predisposition (Romitti *et al.,* 1999; van Rooij *et al.,* 2001). Specifically, there has been a search for susceptibility genes which, in and of themselves, increase the risk of a particular malformation. Susceptibility genes may interact with teratogens, resulting in an even greater risk of a specific malformation (gene-environment interaction). This susceptibility gene may be nonspecific, such as the ability or inability to metabolize a teratogen to a nontoxic metabolite, or conversely, to a more toxic metabolite (Buehler *et al.,* 1990). Secondly, there may be gene or gene products that are specifically involved in a particular embryonic event that are impacted differently by a teratogen depending upon the gene variant.

Malformations comprise a large number of different anomalies (e.g. clubbed feet, cleft lip, transposition of the great vessels of the heart, etc.) and there may be several etiologies for the same malformation. Therefore, large studies looking at smoking may not detect an overall rise in incidence of a particular malformation associated with a given etiology, even though smoking may be associated with a doubling of the occurrence of malformations associated with a particular etiology. Furthermore, certain individuals may be both unable to detoxify a teratogen while also carrying a susceptibility gene variant that, when combined with the teratogen, results in the malformation. Thus, there may be a large increase in the incidence of a specific malformation among individuals with a particular genetic make up that may not be detectable in epidemiology studies.

Since the previous review, there have been little additional data regarding ETS exposure of non-smoking pregnant women and the risks of congenital malformations. The Surgeon General's report noted equivocal findings regarding maternal smoking and the risks of congenital malformations (U.S.DHHS, 2001). Studies that look at malformation rates for large numbers of

births have found maternal smoking to both increase and decrease the risk of specific malformations.

Susceptibility genes for cleft malformations are an active area of research and there appear to be embryos, based on gene variants, at greater risks of developing cleft malformations if the mother is a smoker. The increased risk of isolated cleft lips and/or palates associated with maternal smoking appears to be due to a gene environment interaction. A number of candidate susceptibility genes has been identified, although there is a disagreement in the literature about this. Particular variants of these genes, when combined with maternal smoking, are associated with an increased risk of cleft malformation. Clefts are highly visible malformations and are one of the most common malformations, occurring in one in one thousand births. Visibility and commonness facilitate detection. Other malformations that may be impacted by smoking include single ventricles of the heart, anal atresia, limb abnormalities, gastroschisis and neural tube defects.

3.4.1. Human Studies of Congenital Malformations and ETS Exposure

Studies that have examined the potential association of prenatal ETS exposure and congenital malformations are given below. Generally maternal ETS exposure is based on paternal smoking status only. Thus any association seen may be due to a direct effect of smoking on sperm, rather than due to ETS exposure of the mother. Some studies have suggested that active smoking might cause genetic damage to the sperm as reflected by alterations in sperm parameters (Evans *et al.,* 1981; Marshburn *et al.,* 1989). Although little work has been done associating sperm parameters with pregnancy outcome, genetic damage could theoretically lead to a birth defect. Given the controversial nature of the data on the association of maternal active smoking and congenital malformations, we also present those results with the studies reviewed that looked at both maternal and paternal smoking.

Anal Atresia *(Yuan et al,. 1995)*

This is a Japanese case control study of anal atresia (both syndromic and isolated), which utilized a birth registry of 216,707 births and stillbirths between 1989 and 1994. There were 84 cases of anal atresia and 174 controls. Controls were selected from the same birth registry and did not

have a malformation. The two consecutive births after the case that were matched to the case with respect to maternal age, sex, parity, and season of birth were selected. The methods for collecting parental smoking and drinking habit data were not given. Neither parent was exposed to specific chemicals or physical factors at work. Maternal ETS exposure was not associated with an increased risk of anal atresia. There was a non-significant increase in risk of anal atresia if the mother was a smoker and a significant increase in risk if the mother drank during the first trimester (OR 4.8; 95% CI 1.2; 19.1). The strength of this study is the high prevalence of smoking among the fathers (approximately 50%) and the low prevalence of smoking among the mothers (approximately 10%). As with many studies of specific malformations, this study may be too small to detect differences in risks. This study does not support an increased risk of anal atresia associated with maternal ETS exposure.

Oral Clefts *(Shaw et al., 1996)*

This is a California case control study of oral cleft (clefts of the lips, palate or both) identified by the California Defects Monitoring Program. Control cases were drawn from the same county as the case, had a similar time of birth and had no reportable malformations during the first year of life. Otherwise, controls were not matched to cases. Mothers were interviewed three to four years after delivery regarding maternal and paternal smoking habits and ETS exposure prior to conception and during the first trimester. There were 731 cases and 734 controls. There was an increased risk of isolated oral cleft if the mother was a smoker. This risk was higher if the father was a smoker and the risks increased further if the baby carried one or two copies of the A2 allele for the TGFα gene (transforming growth factor-alpha).

Among non-smoking mothers there was no increase in the risk of a cleft defect if the father was a smoker or the mother was exposed to ETS. But, if the baby carried the A2 allele for TGFα, the risk of a cleft for a fetus of a maternal non-smoker was similar to that of maternal smokers whose babies carry the A2 allele. Specifically, among smoking mothers the OR for isolated clefts ranged from 2.1 to 2.8 (range of 95% CI 1.1; 7.2) depending upon the number of cigarettes smoked and the smoking status of the father. If the baby of the smoking mother carried an A2 allele for TGFα, the OR for isolated cleft lips with or without a cleft palate was 6.1 (95% CI 1.1; 36.6) and the OR for isolated cleft palates was 9 (95% CI 1.4; 61.9). Among non-smoking

women exposed to ETS whose babies carried the TGFα-A2 allele, the risk of isolated cleft lip ± isolated cleft palate was 9.8 (95% CI 1.1; 218) and the risk of isolated cleft palates was 5.3 (95% CI 0.55; 124).

It does not seem plausible that smoking twenty cigarettes per day by a mother during the first trimester has approximately the same risk as ETS exposure during the first trimester. Possibly, the A2 allele, independent of smoking or ETS exposure, is responsible for the increased risk. On the other hand, the number of cases with the A2 allele was small and smoke exposure was determined retrospectively after three to four years, making recall bias a strong possibility. In addition, research has shown that when interviewed postpartum, mothers of babies that had fetal distress during delivery decreased their report of smoking when compared to smoking status data obtained during prenatal care visits (Wong, 2001), while mothers who had uneventful deliveries did not.

Oral Clefts *(Romitti et al., 1999)*

This is a population based case control study of 366 cases of cleft lips and palates identified through the Iowa Birth Defects Registry (1987-1994) and 393 controls without malformations. Data were collected regarding paternal smoking habits, as well as maternal smoking and drinking habits. Maternal smoking was associated with an increased risk of cleft palates (OR 2.3; 95% CI 1.1; 4.6) compared to non-smokers; and this risk was higher for male infants than females. Paternal smoking was not associated with an increased risk of cleft.

Cardiac Malformations *(Steinberger et al., 2002)*

This is a population based case control study of 55 cases of single ventricle type cardiac malformations derived from the Baltimore Washington Infant Study (BWIS) of cardiovascular malformations (1981-1989). Control infants (N=3572) did not have cardiac defects and were randomly selected from the regional cohort of live births. Paternal cigarette smoking and paternal alcohol consumption were associated with all cases of single ventricle malformations.

3.4.2. Malformations, Discussion and Conclusions

Given that the results of studies of active smoking have been inconsistent and the Surgeon General's report stated that there was no association between congenital malformations and active smoking (U.S. DHHS, 2001), a teratogenic effect of ETS is unlikely to be strong. It would be very difficult to detect a significant association of a weak teratogen, with outcomes as rare as specific birth defects. Furthermore, because of the relative dearth of information on causes of malformations, it is difficult to determine whether confounding variables have been adequately controlled. Indeed, in the previous document it was concluded that "*it is not possible at this time to determine whether there is an association of ETS exposure and birth defects.*" This conclusion remains unchanged by recent studies.

There are eleven studies that have investigated congenital malformations and maternal ETS exposure, six of which have been published since the previous monograph (Cal/EPA 1997). In almost all studies, paternal smoking is used as a surrogate marker for ETS exposure. Windham noted (Cal/EPA 1997) that epidemiologic studies suggest a moderate association of severe congenital malformations with paternal smoking, although none of the research presented compelling evidence that ETS exposure caused congenital malformations. The use of paternal smoking status as a surrogate for ETS exposure means that a direct effect of active smoking cannot be ruled out.

Only two studies (Shaw *et al.*, 1996; Wasserman *et al.,* 1996) investigated ETS exposure independent of paternal smoking. Shaw *et al.* found an increased risk of oral clefts (OR range = 9-9.8) in babies of non-smokers with ETS exposure if the baby carried the TGFα A2 allele. ETS-exposed non-smokers and active smokers had similar elevations on risk associated with TGFα A2 allele (OR range 6.1 – 9.0). It does not seem biologically plausible that active smoking and ETS exposure carry the same risk for isolated clefts in the presence of the TGFα A2 allele.

Two investigators studied the association between oral clefts and parental smoking (Shaw *et al.*, 1996 and Romitti *et al.*, 1999). One study (Shaw *et al.*, 1996) found an increased risk of clefts if the mother was a non-smoker with ETS and the baby carried the TGFα A2 allele but otherwise there was no increase in risk of cleft in maternal non-smokers exposed to ETS.

The risks of various kinds of cardiac malformations were investigated in two studies (Wasserman *et al.*, 1996 and Steinberger *et al.*, 2002). The study by Wasserman found a significant increase in risk of tetralogy of Fallot associated with ETS exposure in non-smokers, although it was one of thirty odd ratios calculated for non-smokers with ETS, and it was the only one that was significantly elevated. The study by Steinberger (2002) found that all cases of single ventricle, a rare type of cardiac defect, were associated with paternal smoking and paternal alcohol consumption. These studies do not provide compelling additional data for an association between maternal ETS exposure and cardiac defects.

Only one study not included in the previous monograph investigating neural tube defects (NTD) was located. This study (Wasserman *et al.*, 1996) found no increased risk or a non-significant increase in risk of NTD associated with parental smoking.

A variety of other malformations are presented in the synopses: multiple malformations, severe defects, major defects, minor defects, urethral stenosis, anal atresia and limb defects. In the previous monograph, Mau and Netter (1974) found a significant elevation in risk of severe malformations associated with paternal smoking. Otherwise all of these investigations found no elevation of risk or a non-significant elevation in risk associated with paternal smoking or ETS exposure of maternal non-smokers.

Facial clefts, cardiac malformations, and defects of the nervous system (CNS, NTD) are common congenital defects, irrespective of exposure to toxins such as tobacco smoke. The data presented here do not support an increased risk of congenital malformation associated with ETS exposure in selected populations. The etiology of malformations is just beginning to be unraveled. Over the past decade the percentage of malformations classified as idiopathic has decreased from approximately 70% to 55% as some malformations are found to have a genetic etiology.

Although the research presented here does not support an association between maternal ETS exposure and an increased risk of congenital malformations, these data should not be construed to mean that there is no increased risk of congenital malformations associated with maternal ETS exposure. Just as there appears to be a gene environment interaction between BW and maternal smoking (Wang *et al.*, 2000), there may be gene-environment interactions for congenital

malformations. It will be difficult to demonstrate a gene environment interaction for congenital malformation because there may be multiple etiologies for the same malformation, and there are so many malformations.

3.5. Animal Studies of Tobacco Smoke Exposure

There is a limited number of animal studies of mainstream and sidestream smoke. Data from the studies published since the previous monograph are given in Table 3.8. Animals exposed to tobacco smoke inhale the smoke as humans do, but smoke particulate matter also may deposit on their fur. Unlike humans, animals groom their fur by licking it, thus they may also ingest tobacco smoke particulate matter.

Information on perinatal mortality in animals is provided by endpoints such as numbers of resorptions, numbers of live and dead fetuses at term (in studies with term hysterectomy), and litter size (in studies with spontaneous birth). Studies using mainstream smoke presented in the previous monograph were not generally supportive of effects on these parameters. In the three available studies using sidestream smoke (SS), one study (Witschi *et al.,* 1994) found statistically significant effects of SS exposure on both the number of implantation sites per litter and the number of live pups per litter; this suggests that the primary effect was on implantation. The other two studies (Leichter, 1989; Rajini *et al.*, 1994) did not find effects of SS exposure on variables related to perinatal mortality. No new studies examining perinatal mortality in animals were identified.

Regarding the association between fetal malformation and ETS exposure in animals, the previous monograph stated:

> "Malformations in animals are detected in term fetuses by gross examination, soft tissue examination via dissection and skeletal examination after staining; a complete teratology study includes all three exams. Of seven studies of mainstream smoke using one or more of these techniques, four did not find any effects (Wagner et al., 1972; Reznik and Marquard, 1980; Peterson et al., 1981; Bassi et al., 1984) and three mentioned limited findings (Schoeneck, 1941; Tachi and Aoyama 1983; Amankwah et al., 1985) but did not provide enough information for evaluation or for characterization of defects. Of the three

available sidestream smoke studies, one (Witschi et al., 1994) did not examine malformations. Using gross examination only, Leichter (1989) reported no effects. Rajini et al. (1994) reported finding no effects using gross and skeletal examinations, but did no soft tissue examination. Thus no complete teratology study has been conducted with sidestream smoke."

Table 3.8: Animal studies of mainstream or sidestream smoke

Reference	Animal	Gestational Cigarette Smoke Exposure Findings:
Jalili et al. 1998	Mice	Increased number of DNA deletions in mouse embryo.
Ji et al. 1998	Rats	Maternal prenatal exposure to aged and diluted sidestream smoke: No effect on fetal weight; there was a significant alteration in the developmental expression of pulmonary Clara cells.
Florek *et al.* 1999a	Rats	Decreased maternal weight, delayed lung maturation in offspring.
Florek *et al.* 1999b	Rats	Three-generation study of fertility and reproduction. No significant differences found although there was a trend for a decrease in the number of pregnancies, in the mating index, and in the fertility index. At high cigarette smoke levels, this study approximated active smoking. At levels more consistent with ETS exposure, no differences were found.
Nelson *et al.* 1999a	Rats	Dose dependent reduction in birth weight. No macroscopic malformations. Widespread retardation of ossification.
Nelson *et al.* 1999b	Rats	Histopathologic changes noted in bronchial muscles, liver, kidneys, stomach, and intestines.
Czekaj et al. 2000	Rats	The effect of tobacco smoke exposure on fetal rat CYP2B1 expression.
Elliot et al. 2001	Guinea pigs	Increased airway responsiveness, alteration in alveolar attachment points.
Slotkin et al. 2001	Rat	Increased adenylyl cyclase activity in brain and heart. Inhibition of coupling of beta adrenergic receptors to adenylyl cyclase in brain. Decrease in muscarinic - m2 receptor expression in heart. Level of prenatal ETS exposure consistent with active smoking.
Hasan et al. 2001	Rats	Selective reduction of fetal protein kinase C and nitrate oxide synthetase in dorsocaudal brain stem.

No complete teratology studies conducted with sidestream smoke were found for this update. The recent animal studies summarized in Table 3.8 focused on histologic and/or biochemical end points. Among these a study by Nelson *et al.* (1999a) reported an increase in the rate of apoptosis in several tissues from fetuses after maternal exposure to sidestream smoke. This observation is consistent with their other report (Nelson *et al.*, 1999b) of decrements in fetal

weights and intrauterine growth following smoke exposure and may suggest a mechanism for IUGR in human fetuses similarly exposed.

3.5.1. Animal Studies – Conclusion

The animal data presented in Table 3.8 do not materially affect the conclusions based on data in humans. Those studies that reported histologic and biochemical changes associated with exposure to tobacco smoke support the studies of prenatal exposure to parental nicotine (Dempsey and Benowitz, 2001).

3.6. Final Summary

In summary, data presented here indicate that ETS exposure of non-smoking pregnant women is associated with a 20 to 100 g decrease in BW. This is in agreement with that reported in the previous document, although the magnitude of the effect is larger, and strengthens the conclusion that ETS may be causally associated with decreases in BWs. This may be viewed by some as a modest reduction in BW, however, it is a mean value and may indicate a downward shift in the BW distribution curve so that there is an increase in the number of babies that are growth retarded. Data presented here indicate that there is a downward shift in the distribution as evidenced by an increase in the risk of delivering a growth-retarded baby (LBW, SGA, SFD or IUGR) associated with ETS exposure of non-smoking pregnant women (Table 3.A below). Indeed, the more recent studies are more strongly supportive of a causal association between ETS exposure and restricted fetal growth and PTD than was seen in the previous document. Recent research has demonstrated gene environment interactions involving cigarette smoking and drug metabolizing enzymes. Based upon genetic differences in drug metabolizing enzymes, subgroups of fetuses appear to be at much greater risk of adverse outcomes associated with maternal smoking, specifically increased risks of LBW and PTD. Similarly there appear to be subgroups of fetuses, which are more susceptible to the effects of maternal ETS exposure.

Birth weight decrements may also be a surrogate indicator for other fetal abnormalities. Research (Dempsey and Benowitz, 2001) has shown a myriad of molecular biologic differences in the mother, newborn and placenta associated with maternal smoking. Similar differences may be found between ETS exposed and ETS unexposed pregnant non-smokers. Consistent with the previous document, the limited data presented here do not support an increase in risk of

pregnancy wastage associated with maternal ETS exposure or an increased risk of congenital malformations. Future research may be able to determine if there are subgroups that are at increased risk of pregnancy wastage or malformations based on genetic predisposition.

Table 3.A: ETS and Outcome: LBW, SGA, SFD, IUGR and PTD

Reference Date	Total N	MNS[1] no ETS	MNS[1] w/ETS	OR, RR (95% CI) for IUGR, LBW, SGA, SFD and PTD[2]	Confounders and Covariates Adjustments[3]
Underwood *et al.*, 1967	48,505	9,427	15,233	LBW : 0.9 (0.8,; 1.0) PS≥ 30CPD: 1.05 (0.82; 1.3) PTD: 0.9 (0.8; 1.0) PS≥ 30 CPD: 1.05 (0.8; 1.3)	Sex, MWt, ExAS
Yershalmy *et al.*, 1971	13,083	8,286		LBW: 0.9 n.s.	Oth, ExAS
Mau & Netter *et al.*, 1974	5,183	2,070	1,626	LBW: 1.4 (1; 1.9) PTD: 1.2 (0.9; 1.7)	None reported
Martin *et al.*, 1986	3,891	1,707	906	LBW OR 2.2 (1.1; 4.5) PTD n.s.	GA, MA, Eth, Alc, Drg, SES, MWt, MHt, Oth, ExAS
Haddow *et al.*, 1988	1,231	376	855	LBW: RR 1.29 – no statistics	Eth, Par, MWt, MHt, Oth, ExAS
Nakamura *et al.*, 1988	2,005	561	1,444	LBW: 1.4 (0.9; 2.2) SGA: crude 1.2 (0.8; 2.0) PTD: crude 1.2 (0.8; 1.8)	GA, MA, Par, Alc, SES, Oth, ExAS
Chen *et al.*, 1989	1,162	325	837	LBW: 1.5 (0.75; 3.2)	Sex, Par, SES, Oth, ExAS
Saito 1991	2,713	1,311	1,402	SFD: 1.3; $p<0.05$ PS>20CPD: 1.4; $p<0.05$ PTD: n.s.	
Ogawa *et al.*, 1991	5,336	3,606	1,730	LBW: 1.0 (0.7; 1.5)	GA, MA, Par, Alc, MHt, Oth, ExAS
Ahlborg & Bodin 1991	4,701	2,170	1,703	High ETS - LBW: 1.4 (0.3; 5.9) High ETS – SAB: 2.2 (1.2; 3.8)	GA, Sex, Par, Alc, Oth
Mathai *et al.*, 1992	994	474	520	LBW: 1.0 (0.4, 2.3) PTD: 1.6 (0.8; 2.9)	GA, MA, Sex, Par, SES, MHt, Oth
Zhang & Ratcliffe 1993	1,765	1,033	732	LBW: 1.07, n.s. IUGR: 1.1, n.s.	GA, Sex, Par, MHt, Oth, ExAS
Fortier *et al.*, 1994	> 7,000	2,368	2,276	IUGR: 1.1 (0.85; 1.4) PTD: 0.98 (0.56; 1.73)	Par, MWt, Oth
Mainous & Hueston 1994	3,253	743	2,510	LBW: 1.6 (0.92; 2.7) high ETS LBW: 2.3 (1.1; 5.0)	Eth, Par, SES, Oth, ExAS
Eskenazi *et al.*, 1995	2,292	2,129	114	LBW: 1.35 (0.6; 3.0)	GA, MA, Eth, Par, Alc, MWt, MHt, Oth
Chen *et al.*, 1995	225	100	120	IUGR: 0.5 (0.14; 1.7)	Eth, Par, Alc, Drg, SES, MWt, Oth
Jedrychowski & Flak 1996	1,115	452	512	LBW: 1.46 (0.83; 2.6)	GA, Sex, Par

[1] MNS: maternal non-smoker (Blank – number not given); [2] CPD: cigarettes per day; IUGR: intrauterine growth restriction; LBW: low birth weight; PTD: preterm delivery; SFD: small for date; SGA: small for gestational age. [3] Abbreviations: ALC: alcohol use; Drg: drug use; Eth: ethnicity; ExAS: excludes active smokers; GA: gestational age; MA: maternal age; MHt: maternal height; MWt: maternal weight; Oth: other; Par: parity; SES: socioeconomic status; Sex: sex of the newborn.

Table 3.A: ETS and Outcome: LBW, SGA, SFD, IUGR and PTD (continued)

Reference Date	Total N	MNS[1] no ETS	MNS[1] w/ETS	OR, RR (95% CI) for IUGR, LBW, SGA, SFD and PTD[2]	Confounders and Covariates Adjustments[3]
Ahluwalia *et al.*, 1997	17,412	10,639	2,855	LBW: <30yo 0.97 (0.76; 1.23) ≥30yo 2.4 (1.5; 3.9) PTD: <30yo 0.9 (0.8; 1.1) ≥30yo 1.9 (1.2; 2.9) SGA: <30yo 0.97 (0.8; 1.3) ≥30yo 1.3 (0.8; 2.2)	Eth, Par, Alc, MWt, Oth
Horta *et al.*, 1997	5,166			LBW: 1.18 (0.94; 1.48) PTD: 1.25 (0.99; 1.57) IUGR: 1.33 (1.05, 1.68)	GA, MA, Eth, Par, SES, MWt, MHt, Oth
Nafstad *et al.*, 1998	163	68	54	SGA: 1.0 (0.4; 2.1)	GA, Sex, MWt, MHt, Oth
Dejin-Karlsson *et al.*, 1998	854	247	345	SGA: 3.9 (1.4; 10.7)	GA, MA, Eth, Par, Alc, Drg, SES, MWt, MHt, Oth
Windham *et al.*, 1999	992			LBW 1.0 (0.52; 2.1) Term LBW 1.8 (0.64; 4.8) SGA 1.4; (0.79; 2.5)	GA, Eth, Alc, Oth
Windham *et al.*, 2000	4,099	2,887	759	high ETS LBW 1.8 (0.82; 4.1) high ETS PTD 1.6 (0.87; 2.9) high ETS very PTD 2.4 (1.0; 5.3) Ethnicity not caucasian high ETS LBW 3.8 (1.5; 9.8) high ETS PTD 2.4 (1.1; 5.5) high ETS very PTD 3.8 (1.3; 10.7) Mat age >30y, PTD 2.8 (1.2; 6.6)	GA, MA, Eth, Par, Alc, SES, MWt, MHt, ExAS
Kharrazi *et al.*, 2001	2,796	951	1,845	Adverse Outcome 1.36 (1.06; 1.72) LBW: 1.42 (0.91; 2.21) PTD: 1.29 (0.97; 1.72)	GA, Sex, Eth, SES, Oth, ExAS
Jaakkola *et al.*, 2001	477	288	233	LBW: 1.51 (1.02; 2.26)	ExAS
Dejmek *et al.*, 2002	6,866	3,710	1,797	LBW: 1.51 (1.02; 2.26) IUGR: 1.08 (0.79; 2.5)	Sex, Eth, Par, Alc, SES, MWt, MHt, Oth

[1] MNS: maternal non-smoker (Blank – number not given); [2] CPD: cigarettes per day; IUGR: intrauterine growth restriction; LBW: low birth weight; PTD: preterm delivery; SFD: small for date; SGA: small for gestational age. [3] Abbreviations: ALC: alcohol use; Drg: drug use; Eth: ethnicity; ExAS: excludes active smokers; GA: gestational age; MA: maternal age; MHt: maternal height; MWt: maternal weight; Oth: other; Par: parity; SES: socioeconomic status; Sex: sex of the newborn.

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